# Distinct in vivo roles of secreted APP ectodomain variants APPs  and APPs  in regulation of spine density, synaptic plasticity, and cognition

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Abstract: Increasing evidence suggests that synaptic functions of the amyloid precursor protein (APP), which is key to Alzheimer pathogenesis, may be carried out by its secreted ectodomain (APPs). The specific roles of APPs  and APPs  fragments, generated by non-amyloidogenic or amyloidogenic APP processing, respectively, remain however unclear. Here, we expressed APPs  or APPs  in the adult brain of conditional double knockout mice (cDKO) lacking APP and the related APLP2. APPs  efficiently rescued deficits in spine density, synaptic plasticity (LTP and PPF), and spatial reference memory of cDKO mice. In contrast, APPs  failed to show any detectable effects on synaptic plasticity and spine density. The C-terminal 16 amino acids of APPs  (lacking in APPs ) proved sufficient to facilitate LTP in a mechanism that depends on functional nicotinic  7-nAChRs. Further, APPs  showed high-affinity, allosteric potentiation of heterologously expressed  7-nAChRs in oocytes. Collectively, we identified  7-nAChRs as a crucial physiological receptor specific for APPs  and show distinct in vivo roles for APPs  versus APPs . This implies that reduced levels of APPs  that might occur during Alzheimer pathogenesis cannot be compensated by APPs .

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**Distinct *in vivo role* of APPsα versus APPsβ for spine density, synaptic plasticity and cognition**

**Running Title:**

Functions of APPsα and APPsβ

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Alzheimer, amyloid precursor protein, soluble APPsα, synaptic plasticity, nicotinic acetylcholine receptor, spines, cognition

## Abstract

Increasing evidence suggests that the synaptic functions of the amyloid precursor protein (APP), that is key to Alzheimer pathogenesis, may be carried out by its secreted ectodomain. However, the specific role of APPs fragments generated by non-amyloidogenic or amyloidogenic APP processing remain unclear. Here, we expressed APPsα or APPsβ in the adult brain of conditional double knockout mice (cDKO) lacking APP and the related APLP2. APPsα efficiently rescued deficits in spine density, synaptic plasticity (LTP and PPF) and spatial reference memory of cDKO mice. In contrast, APPsβ failed to show any detectable effects on synaptic plasticity and spine density. The C-terminal 16 amino acids of APPsα (lacking in APPsβ) proved sufficient to facilitate LTP in a mechanism that depends on functional nicotinic α7-nAChRs. Further, APPsα showed high-affinity, allosteric potentiation of heterologously expressed α7-nAChRs in oocytes. Collectively, we identified α7-nAChRs as a crucial physiological receptor specific for APPsα and show distinct *in vivo* roles for APPsα versus APPsβ. This implies that reduced levels of APPsα that might occur during Alzheimer pathogenesis cannot be compensated by APPsβ.

## Introduction

Alzheimer's disease (AD) is characterized by the accumulation of β-amyloid peptides (Aβ), that are derived from the amyloid precursor protein (APP) by proteolytic cleavage (Selkoe & Hardy, 2016). Two principal physiological pathways either prevent or promote Aβ generation. Within the amyloidogenic pathway APP processing is shifted towards the production of Aβ and secreted APPsβ, by consecutive β-secretase (BACE1) and γ-secretase cleavage (Vassar et al, 2014). In AD BACE1 is upregulated, favoring amyloidogenic APP processing (Ahmed et al, 2010; Holsinger et al, 2002). In the alternative, non-amyloidogenic pathway cleavage of APP within the Aβ region by the major α-secretase ADAM10 (a disintegrin and metalloprotease) prevents Aβ generation and liberates APPsα, that is secreted into the extracellular space (Saftig & Lichtenthaler, 2015). Shifting APP processing towards non-amyloidogenic processing has therefore been suggested as a therapeutic strategy for AD (Mockett et al, 2017). An important and still unresolved question is whether in addition to neurotoxic Aβ accumulation a concomitant reduction in APPsα level, or an altered APPsα/APPsβ ratio may contribute to AD symptoms and pathogenesis (reviewed by Mockett et al, 2017). In this regard, it will be crucial to know whether APPsα and APPsβ, that is only 16 aminoacids shorter than APPsα, serve largely similar or distinct, possibly even opposite physiological functions. While our previous studies and work from others indicated that APPsα has neurotrophic and neuroprotective effects, including synaptogenic, LTP facilitating and memory enhancing properties (Hefter et al, 2016; Hick et al, 2015; Meziane et al, 1998; Milosch et al, 2014; Plummer et al, 2016; Ring et al, 2007; Taylor et al, 2008; Weyer et al, 2014), only few and mostly conflicting studies have as yet addressed the functions of APPsβ (Chasseigneaux & Allinquant, 2012; Li et al, 2010; Nikolaev et al, 2009; Weyer et al, 2011). So far, the molecular basis of any difference compared to APPsα remained unclear. Thus, a more detailed knowledge about the specific functions of the secreted APP ectodomains is essential to understand AD pathogenesis and evaluate risks of ongoing pharmacotherapy, as well as to elucidate APP physiology.

There is a large body of evidence indicating that APP family proteins are multimodal proteins that can function as ligands via their secreted fragments or as cell surface proteins important for synaptic adhesion and signal transduction (Müller et al, 2017). Major insights into the physiological functions of APP and the related APLPs (APP like proteins) were obtained from knockout models (Müller et al, 2017). While most impairments of APP-KO mice emerged only in aged mice (Dawson et al, 1999; Lee et al, 2010; Ring et al, 2007; Seabrook et al, 1999; Tyan et al, 2012), combined APP/APLP2 double knockout mice die shortly after birth, likely due to severe deficits at the neuromuscular junction (Heber et al, 2000; Klevanski et al, 2014; von Koch et al, 1997; Wang et al, 2005). Recently generated forebrain-specific double knockout mice (termed NexCre cDKO), that lack APP from embryonic day 11.5 onwards in

excitatory forebrain neurons on a global constitutive APLP2-KO background, showed a severe synaptic phenotype already at young age, including reduced spine density and impaired LTP in the hippocampus, as well as deficits in learning and memory (Hick et al, 2015). Interestingly, the LTP impairment of NexCre cDKO mice could be ameliorated by acute APPsα application onto brain slices *in vitro*, while the molecular mechanism and receptor(s) mediating its function remained unknown (Hick et al, 2015).

More recently, we showed that AAV-mediated intracranial expression of APPsα can mitigate the Aβ related synaptic deficits of APP/PS1 mice *in vivo* (Fol et al, 2016). Intracranial AAV-APPsα injections enhanced spine density, improved LTP deficits and memory, but at the same time also reduced soluble Aβ levels and plaque load, likely due to enhanced Aβ clearance (Fol et al, 2016). In addition, APPsα had been reported to lower Aβ by directly binding to and inhibiting BACE (Obregon et al, 2012). These results raised the question of whether the beneficial *in vivo* effects of APPsα are mainly due to its Aβ lowering properties.

Here, we asked whether APPsα may also have synaptotrophic effects in an Aβ independent pathology with synaptic impairments and used viral vectors to express APPsα intracranially in NexCre cDKO mice. Moreover, we set out to compare side-by-side the properties of APPsα and APPsβ *in vivo*. We demonstrate that APPsα is sufficient to fully rescue hippocampal spine density, to restore LTP and partially rescue spatial memory in adult NexCre cDKO mice. In sharp contrast, despite similar expression level, APPsβ failed to show any detectable effects on synaptic plasticity and spine density. Finally, we show that the CTα16 domain of APPsα (that is lacking in APPsβ) is able to facilitate LTP to the same extent as APPsα, in a mechanism that involves functional nicotinic α7 acetylcholine receptors (α7-nAChRs). Moreover, we show that nanomolar concentrations of APPsα (but not APPsβ) can directly potentiate α7-nAChRs-mediated currents upon heterologous expression in *Xenopus* ooctyes and increases the apparent agonist affinity as a positive allosteric modulator. Collectively, our analysis identifies the α7-nAChR as a crucial physiological receptor for APPsα and reveals distinct *in vivo* roles of APPsα versus APPsβ.

## Materials and Methods

### Mice

Experiments on animals were performed in accordance with the guidelines and regulations set forth by the German Animal Welfare Act and the Regierungspräsidium Karlsruhe, Germany. Generation and genotyping of NexCre cDKO mice (further referred as cDKO mice) were as described previously (Hick et al, 2015). Genotype of experimental animals: NexCre cDKO (cDKO), $APP^{flox/flox}APLP2^{-/-}NexCre^{+/T}$ and littermate controls (LM controls), APP-WT (=$APP^{flox/flox}$)$APLP2^{-/-}$.

### AAV plasmid design and vector production

The mouse APPsα coding sequence (derived from Uniprot: P12023-2) was codon optimized (Geneart, Germany) and then cloned under control of the synapsin promoter into a single-stranded rAAV2-based shuttle vector, as described previously (Fol et al, 2016). Briefly, the bicistronic DNA constructs habour a 2A site that connects the cDNA of lckVenus and muAPPsα. Venus contains a lymphocyte-specific protein tyrosine kinase (lck) derived peptide motif which tethers it to the plasma membrane. For easy detection, an N-terminal double HA-tag was inserted downstream of the APP signal peptide (SP) at the N-terminus of APPsα. The monocistronic control vector, AAV-Venus, encodes only the yellow fluorescent protein Venus. All constructs were packaged into AAV9 capsids. Briefly, viral particles were produced by transient co-transfection of HEK-293 cells with the transfer vector containing the above-mentioned expression cassettes and the helper plasmid pDP9rs. 72 h following transfection, virions were purified and concentrated from cell lysate and supernatant by ultracentrifugation on a iodixanol density gradient followed by buffer exchange to 0.01% pluronic/phosphate-buffered saline (PBS) via a 100 kDa Amicon centrifugal filter unit. Genome copies in the vector stocks were determined by free inverted terminal repeat (ITR)-specific quantitative TaqMan PCR and expressed as genomic copies per µl of concentrated stocks (gc/µl) as described (D'Costa et al, 2016).

### Stereotactic injection of AAVs

Mice were anesthetized by intraperitoneal injection of sleep mix (Medetomedin: 500 µg/kg, Midazolam: 5 mg/kg, Fentanyl: 50 µg/kg in isotonic NaCl solution) and positioned on a stereotactic frame (World Precision Instruments, USA). Vector particles (either AAV-Venus, AAV-APPsα or AAV-APPsβ) were injected into the hippocampus at two injection spots per hemisphere using 1 µl vector stock (titer: 1 x $10^9$ gc/µl) per spot at a rate of 0.2 µl/min. When injection was completed, the cannula was left to rest for 1 min to prevent efflux of viral vector

solution. Stereotactic coordinates of injection sites from bregma were: anteroposterior (A/P): −2 mm, mediolateral (M/L): ±1 mm, dorsoventral (D/V): −2.25 mm and −1.75 mm. Procedures were approved by the local animal care and use committee (35-9185.81/G-304/14, Regierungspräsidium Karlsruhe, Germany)

**Brain samples**

Mice were sacrificed 6 weeks post-injection at 5-6 months of age. Following anesthesia, mice were transcardially perfused with PBS before dissection. For immunohistochemistry, one cerebral hemisphere was dissected and post-fixed in 4% paraformaldehyde (PFA) for 48 h and afterwards stored in PBS at 4°C. 40 µm coronal sections were cut using a vibratome (HM650V Vibratome, Thermo Fisher Scientific, USA) and collected in PBS. The other hemisphere was dissected to segregate hippocampus and cortex for biochemical analysis. Samples were then homogenized using a Polytron homogenizer (Polytron PT-MR 2100, Kinematica AG, Switzerland) in tissue homogenization buffer (THB, 250 mM sucrose, 1 mM EGTA, 20 mM Tris-HCL, pH 7.4) containing 1x protease inhibitor (Complete™ #04693124001, Roche, Switzerland). After centrifugation (10 min, 13,000 rpm, 4°C), the supernatant was collected and the protein concentration was quantified by BCA assay. Lysate aliquots were snap-frozen in liquid $N_2$ and stored at −80 °C. For the detection of the soluble ectodomain fragments APPs, hippocampal lysates were subjected to high-speed centrifugation (60 min at 100,000x g) and membrane-free supernatant was used for further analysis.

**Western blot analysis**

Total brain homogenate or supernatant from ultracentrifugation (10 µg protein) was used for SDS-PAGE. Proteins were separated using 12% Tris-Glycin gels at 20 mA/gel in Laemmli buffer (0.25 M Tris, 2 M glycine and 1% SDS) and transferred to 0.45 µm PVDF membranes (GE Healtcare, USA) using a tank blot at 450 mA for 1 h (for samples after ultracentrifugation: 450 mA for 1.5 h). After blocking in PBS-T (2.5% Tween in PBS) containing 5% (w/v) dried milk powder at room temperature for 60 min, membranes were incubated with the primary antibodies at 4 °C overnight. The following antibodies were used: α-HA-tag (rabbit, 1:1000, #3724, Cell Signaling Technology, USA was used for total hippocampal homogenates or mouse, 1:1000, #2367, Cell Signaling Technology, USA used for analysis after ultracentrifugation), α-GFAP (rabbit, 1:3000, #173002, Synaptic Systems, Germany), α-Iba1 (rabbit, 1:500, #234003, Synaptic Systems, Germany), α-GFP (chicken, 1:1000, #A10262, Thermo Fisher Scientific, USA), α-β-Tubulin (mouse, 1:10000, #MAB3408, Merck Millipore, USA), M3.2 (mouse, 1:1000, kind gift from Paul Mathews), Y188 (rabbit, 1:1000, #ab32136, Abcam, UK). Membranes were then washed with PBS-T, incubated with a

horseradish peroxidase coupled secondary antibody (goat-α-mouse HRP, 1:10000, #115-165-146, Dianova, Germany; donkey-α-rabbit HRP, 1:10000, #711-035-152, Dianova, Germany; goat-α-chicken HRP, 1:10000, #103-035-155, Dianova, Germany) washed again and developed using SignalFire ECL Reagent (#6883, Cell Signaling Technology, USA) or SignalFire™ Elite ECL Reagent (used for analysis after ultracentrifugation, #12757, Cell Signaling Technology, USA). Signals were detected with the BioRad Chemidoc MP imager (BioRad, Hercules, USA) and analyzed using BioRad Image Lab software.

**Immunostaining**

Slices were blocked/permeabilized in blocking buffer (5% BSA, 5% NGS, 0.4% Triton X-100 in PBS) overnight at 4°C. To detect AAV-encoded HA-tagged HA-APPsα, slices were incubated with α-HA antibody (rabbit-α-HA, 1:1000, #3724, Cell Signaling Technology, USA or mouse-α-HA, 1:1000, #H3667, Sigma-Aldrich, USA) overnight at 4°C. After three successive washes with PBS-T (2.5% Tween in PBS) slices were incubated in secondary antibody (goat-α-rabbit Cy3, 1:1500, #711-165-152, Jackson ImmunoResearch Laboratories, USA; goat-α-rabbit Cy5, 1:1500, #111-175-144, Jackson ImmunoResearch Laboratories, USA; donkey-α-mouse Cy3, 1:1500, #715-165-150; Jackson ImmunoResearch Laboratories, USA; goat-α-mouse Cy5, 1:1500, #A10524, Thermo Fisher Scientific, USA). For expression analysis HA-APPsα was coimmunostained overnight with the following primary antibodies: α-NeuN (mouse, 1:1500, #MAB377, Merck Millipore, USA), α-GFAP (rabbit, 1:3000, #173002, Synaptic Systems, Germany) and α-Iba1 (rabbit, 1:500, #234003, Synaptic Systems, Germany). Images were taken with a Axio Observer Z1 (Zeiss, Germany) and a Leica TCS SP5II (Leica, Germany).

**Electrophysiology**

*In vitro* extracellular recordings were performed on acute hippocampal slices of LM controls stereotactically injected with the AAV-Venus (N=5) and cDKO mice injected either with AAV-Venus (N=4), or AAV-APPsα (N=5). In a separate set of experiments cDKO mice were injected either with AAV-Venus (N=5) or AAV-APPsβ (N=5). Investigation of synaptic plasticity was done 7-8 weeks after AAV injection. In-between animals were housed in a temperature- and humidity-controlled room with a 12h light-dark cycle and had access to food and water *ad libitum*.

*Slice preparation*

Acute hippocampal transversal slices were prepared from isoflurane anesthetized individuals. Following decapitation, the brain was removed and quickly transferred into ice-cold carbogenated (95% $O_2$, 5% $CO_2$) artificial cerebrospinal fluid (ACSF) containing

125.0 mM NaCl, 2.0 mM KCl, 1.25 mM $NaH_2PO_4$, 2.0 mM $MgCl_2$, 26.0 mM $NaHCO_3$, 2.0 mM $CaCl_2$ and 25.0 mM glucose. The hippocampus was sectioned into 400 µm thick transversal slices with a vibrating microtome (VT1200S, Leica, Germany) and maintained in carbogenated ACSF at room temperature for at least 1.5 h. Before recording, each slice of the injected animals was inspected for Venus expression in area CA1 and CA3 (Axiovert 35, Zeiss, Germany). Slices lacking fluorescence in the recording areas were excluded from further analysis.

*Peptides and inhibitor*

Acute hippocampal slices of LM controls and cDKO mice were pre-incubated in 30 ml gently carbogenated ACSF containing synthetic CT$\alpha$16 (DAEFGHDSGFEVRHQK) or a scrambled CT$\alpha16_{scr}$ peptide (RFDQHGVEDHAFGESK) of the same aminoacid composition as a control. Recombinant recAPPsα was purified from the supernatant of stably transfected HEK cells as described (Hick et al, 2015) and was used at a concentration of 10 nM, in a custom made incubation chamber for 1 h at RT. Afterwards, slices were transferred into the recording chamber where again 30 ml of ACSF with the respective peptide was circulating in a closed loop during the entire experiment. In another set of experiments, the α7-nicotinic acetylcholine receptor antagonist (α7-nAChR) α-Bungarotoxin (BTX, Merck Millipore, Germany) solved in *Aqua dest.* was used at a final concentration of 10 nM and either co-applied already at the step of pre-incubation (1h before TBS) together with 10 nM CT$\alpha$16 on acute slices or washed in 10 min before baseline recording started.

*Extracellular field recordings*

Slices were placed in a submerged recording chamber and perfused with carbogenated ACSF (32°C, 125.0 mM NaCl, 2.0 mM KCl, 1.25 mM $NaH_2PO_4$, 2.0 mM $MgCl_2$, 26.0 mM $NaHCO_3$, 2.0 mM $CaCl_2$ and 25.0 mM glucose) at a rate of 1.2 to 1.5 ml/min. Field excitatory postsynaptic potentials (*f*EPSPs) were recorded in stratum radiatum of CA1 region with a borosilicate glass micropipette (resistance 2-4 MΩ) filled with 3 M NaCl at a depth of ~150-200 µm. Monopolar tungsten electrodes were used for stimulating the Schaffer collaterals at a frequency of 0.1 Hz. Stimulation intensity was adjusted to ~40% of maximum *f*EPSP slope for 20 minutes baseline recording. LTP was induced by applying theta-Burst stimulation (TBS: 10 trains of 4 pulses at 100 Hz in an 200 ms interval, repeated 3 times).

Basal synaptic transmission properties were analyzed via input-output-(IO) measurements and short-term plasticity was examined via paired pulse facilitation (PPF). The IO-measurements were performed either by application of a defined current values (25-250 µA) or by adjusting the stimulus intensity to certain fiber volley (FV) amplitudes (0.1-0.8 mV). Presynaptic function and short-term plasticity were assessed with the PPF paradigm by

applying a pair of two closely spaced stimuli in inter-stimulus-intervals (ISI) ranging from 10 to 160 ms.

*Data analysis and statistics*

Data of electrophysiological recordings were collected, stored and analyzed with LABVIEW software (National Instruments, USA). The initial slope of *f*EPSPs elicited by stimulation of the Schaffer collaterals was measured over time, normalized to baseline and plotted as average ± SEM. Analysis of the PPF data was performed by calculating the ratio of the slope of the second *f*EPSP divided by the slope of the first one and multiplied by 100. The statistical analysis was performed using Microsoft Excel or GraphPad Prism version 6.0 (GraphPad, USA). Data obtained between two genotypes or two different experimental conditions were compared using an unpaired two-tailed Student's *t*-test. Data including more than 2 different groups were analyzed using a one-Way ANOVA followed by a *post-hoc* Bonferroni´s Test. All data are indicated as mean ± SEM. Values of $p<0.05$ were considered significant and plotted as follows *$p<0.05$, **$p<0.01$, ***$p<0.001$.

*Xenopus Oocyte expression and electrophysiological recording*

The rat α7-nAChR, kindly provided by A. Nicke (Kendel et al, 2013), and the human α1-GlyR (Laube et al, 2000) cDNAs were subcloned into the oocyte expression vector pNKS2 . The original rat α7-nAChR cDNA had been provided by J. Patrick (Baylor College of Medicine, Houston, USA). The rat α7-nAChR or the human α1-GlyR cDNA was linearized and cRNA was synthesized with the SP6 or T7 mMessage mMachine kit, respectively (Ambion, USA). For electrophysiological analysis, *Xenopus laevis* oocytes were injected with 50 ng of the cRNA in a volume of 50 nl. Oocytes were isolated and maintained as described previously (Laube et al, 1997) and approved by the local animal care and use committee (II25.3-19c20/15, RP Darmstadt, Germany). 1-3 days after injection, whole-cell currents were recorded at -70 mV by two-electrode voltage-clamp according to (Laube et al, 2000). Currents were acquired at 200 Hz with a Geneclamp 500B amplifier, a Digidata 1322A digitizer and Clampex 9.2 software (Molecular Devices, USA). The agonists nicotine, acetylcholine or glycine, dissolved in bath solution, was applied either alone for 10 s or after 15 s pre-application of the respective peptides (CTα16, CTα16$_{scr}$, recAPPsα or recAPPsβ) also dissolved in bath solution. All experiments were performed at room temperature.

*Data analysis and statistics*

Currents were measured with Clampfit 9.2 software (Molecular Devices, USA) and results were analyzed using the KaleidaGraph program (Synergy Software, USA) and GraphPad Prism version 6.0 (GraphPad, USA). Peak current responses to nicotine in the absence or presence of APPs$\alpha$ were plotted against agonist concentration and fit with variable slope non-linear regression to establish agonist $EC_{50}$ parameter (Laube et al, 2000). For peptide modulation, responses to $EC_{50}$ nicotine (100µM), acetylcholine (100µM) or glycine (100 µM) after application were analyzed. Normalized CT$\alpha$16-induced fold enhancement of nicotine-induced currents was fit with variable slope non-linear regression (GraphPad Prism version 6.0, GraphPad, USA), yielding $EC_{50}$ parameters for each individual experiment (Laube et al, 1998). All experiments were from at least two batches of oocytes. Data were compared by paired two-tailed Student's t-test. Values of $p<0.05$ were considered significant and plotted as follows *$p<0.05$, **$p<0.01$, ***$p<0.001$. All data are indicated as mean ± SEM.

**Neuronal morphology and spine density**

*Iontophoretic fillings*

In brief, CA1 pyramidal cells were visualized by postfixation filling with Alexa 568 in 200 µm horizontal brain sections of 5-6 months old mice (see Appendix Table S1 for sex of animals). For iontophoretic fillings of CA1 pyramidal neurons, one brain slice at a time was placed in a custom-made chamber filled with cold PBS and visualized on an Olympus BX51WI fixed stage upright microscope (Olympus, Japan). Sharp quartz glass electrodes (Quartz electrodes with filament, O.D: 1.0 mm, I.D.: 0.7 mm, 10 cm length, Sutter instruments, USA) were pulled using the Sutter P-2000 Laser Puller. The tip of the electrode was loaded with 5 mM Alexa 568 dye dissolved in distilled water and backfilled with 0.1 M LiCl dissolved in distilled water. Using a motorized 3D micromanipulator, the electrode was lowered into the hippocampal CA1 region under visual control using a Calcium Crimson filter cube (HQ580/20x, U-Q595LP (339038), HQ630/60m) while applying a negative voltage pulse (−1 V, 1 Hz) to the electrode via a silver wire in line with a 500 MΩ resistor. When piercing of a cell body was observed, the cell was filled by application of a negative 1 Hz current pulse (~5 nA) to the electrode. Filling was for 10 min or until no further filling was observed. Afterwards, slices were fixed for 2 days in 4 % PFA at 4 °C, washed three times in cold PBS and finally mounted in Mowiol.

*Image acquisition*

Images of filled neurons were acquired on a Leica TCS SP5II (Leica, Germany) upright confocal microscope. Overview images of complete neurons used for reconstruction were taken with a 20x objective (Apo 20x/0.75). The complete volume of one filled neuron was imaged with a z-step size of 1 µm and a 2048 x 2048 resolution. Basal and apical segments

were imaged individually with two overlapping stacks. Detailed images of dendritic segments and spines were acquired using a 63x objective (Apo 63x/1.4 oil) and a digital zoom of 5. To fullfill the Nyquist criteria for deconvolution, z-stacks of dendritic segments were captured with a pixel size of 80 nm and a z-step of 130 nm. Laserpower, gain and offset varied from cell to cell as the parameters were always chosen so that the complete range of the grayscale was used. Images for spine counts were deconvoluted with the AutoQuantX3 software (Media Cybernetics, USA).

*Neuronal morphology and spine counts*

Filled CA1 pyramidal neurons were manually reconstructed using the Neurolucida software (MicroBrightField, USA) by an experimentator blind to genotype and injected viral vector. Neurons were only included into Sholl analysis if they showed a completely filled apical or basal tree and well defined dendritic endings. The morphometric Sholl analysis was done using the NeuroExplorer software (MicroBrightField, USA). In short, a series of concentric spheres (centered around the soma) was drawn with an intersection interval of 30 µm and the number of dendrites crossing each sphere, as well as the dendritic length in between each sphere was calculated. This analysis was done separately for basal and apical dendrites and was plotted against the distance from the soma. For evaluation of basal dendritic spine density, at least 3 different randomly chosen dendritic segments of the basal dendritic arbour were imaged. They had to fulfil the following criteria: (1) Lie mostly horizontally to the slice surface, (2) be at least 20 µm away from the soma, (3) have a comparable thickness. The minimum basal dendritic length imaged per neuron was 100 µm. For evaluation of midapical dendritic spine density, at least 3 different dendritic segments of the apical tree were imaged. Midapical was defined as the middle third of the length of the apical dendrite measured from the origin of the apical dendrite from the soma to the endpoint of the tufts. Dendritic segments used for evaluation had to fulfil the following criteria: (1) be of second or third order to assure comparable shaft thickness, (2) lie in the middle third of the main apical dendrite (3) be longer than 10 µm. The minimum midapical dendritic length imaged per neuron was 100 µm. Files in the ND2 format were imported into ImageJ (NIH) using the BioFormats Importer. After adjusting, images were saved in the TIFF format. Dendritic spines were manually counted using the Neurolucida and NeuroExplorer software (MicroBrightField, USA) following the criteria of Holtmaat (Holtmaat et al, 2009) with minor modifications: (1) All spines that protruded laterally from the dendritic shaft and exceeded a length of 0.4 µm were counted. (2) Spines that protruded into the z-plane were only counted if they exceeded the dendritic shaft more than 0.4 µm to the lateral side. (3) Spines that bisected were counted as two spines. (4) Spines had to be at least 10 µm away from branching points and the soma. Spine density was expressed as spines per µm of dendrite.

Prior to statistical analysis and blind to genotype, neurons were excluded if the image quality (poor signal to noise ratio) was not sufficient for counting of spines or for deconvolution.

*Golgi staining*

Golgi staining was done using the Rapid Golgi Staining Kit (FD NeuroTechnologies, USA) according to the manufacturer's instructions. All procedures were performed under dark conditions. One hemisphere of each mouse was used for Western blot analysis and the other hemsiphere for Golgi staining (see Appendix Table S1 for sex of animals). Hemispheres were immersed in 2.5 ml mixtures of equal parts of kit solutions A and B and incubated at room temperature for 2 weeks. After 24 h solution A + B was renewed. Afterwards, brain tissues were stored in solution C at 4°C for at least 72 h, once exchanged after 24 h. Brains were snap-frozen on dry ice and coronal sections of 100 µm were cut with a cryotome (Hyrax C50, Zeiss, Germany). Each section was mounted with Solution C on an adhesive microscope slide pre-coated with 1% gelatin/0.1% chrome alum on both sides and stained according to the manufacturer´s protocol with the exception that RotiClear (Roth, Germany) was used instead of xylene. Finally, slices were cover-slipped with Permount (Thermo Fisher Scientific, USA).

*Imaging and analysis of spine density after Golgi staining*

Imaging of second- or third-order dendritic branches of hippocampal pyramidal neurons of area CA1 was done with an Axio Observer Z1 (Zeiss, Germany) for Golgi-stained neurons using a 63x oil objective. Z-stack thickness was hold constant at 130 nm. The number of spines was determined per micrometer of dendritic length (in total 100 µm per neuron) at apical and basal compartments using Neurolucida software (MicroBrightField, USA). Spines in the area around branching points and the soma were excluded from analysis. Five animals per genotype and 3-4 neurons per animal were analysed blind to genotype and injected viral vector.

**Behavioral analysis**

*Animals*

Mice (cDKO + AAV-APPsα: 9M + 9F, cDKO + AAV-Venus: 8M + 10F, LM control + AAV-Venus: 12M + 10F) were housed under a 12/12h light-dark cycle (lights on at 20:00) in groups of 2-5, unless individual housing was required by experimental protocols or to prevent fighting. Testing occurred during the dark phase under dim light (approximately 12 lux). Mice were transferred to the testing room 30 min before testing. Procedures were approved by the Veterinary Office of the Canton of Zurich (license 44/2015).

*Behavioral procedures*

Morris Water Maze place navigation. The round white poly-propylene pool had a diameter of 150 cm with 68 cm high walls. It was filled with water (24-26° C, depth 15 cm), rendered opaque by addition of 1 l of milk (UHT whole milk 3.5% fat, Coop, Switzerland). The white quadratic goal platform (14x14 cm) was made of metallic wire mesh. It was hidden 0.5 cm below the water surface in the center of one of the 4 quadrants, 30 cm from the side wall. Salient extra-maze cues were placed on the walls of the testing room. Animals performed 30 training trials (max. duration 120 s), 6 per day with intertrial intervals of 30-60 min and varying starting positions. During the first 18 trials the hidden platform was held in the same position (acquisition phase) and then moved to the opposite quadrant for the remaining 12 trials (reversal phase). The first 60 s of the first reversal trial served as probe trial to test for spatial retention. Time spent in a circular target zone comprising 12.5% of the pool surface was compared with corresponding control zones in adjacent quadrants.

*Data analysis and statistics*

The path of moving mice was tracked using Noldus EthoVision 3.1 (Noldus, Netherlands). For analysis all data were imported in custom programmed software Wintrack (www.wintrack.ch; Wolfer et al, 2001). Data were evaluated using an ANOVA model with genotype (cDKO + AAV-APPs$\alpha$, cDKO + AAV-Venus, LM control + AAV-Venus) and sex as between subject factors. Within subject factors were added as needed to explore the dependence of genotype effects on place or time. Significant interactions and where necessary significant main effects were further explored by pairwise t-tests or by splitting the ANOVA model, as appropriate. Variables with strongly skewed distributions or strong correlations between variances and group means were subjected to Box-Cox transformation before statistical analysis. The significance threshold was set at 0.05. The false discovery rate (FDR) control procedure of Hochberg was applied to groups of conceptually related variables within single tests to correct significance thresholds for multiple comparisons. Similarly, FDR correction was applied during *post-hoc* testing. As genotype and treatment effects were independent of sex, the sex factor is not reported in results and figures. Statistical analyses were run using R version 3.2.3 complemented with the packages psych and moments.

*For further methods related to EV Figures see Appendix Supplementary Methods*

## Results

**AAV-APPsα injection mediates efficient and neuron specific expression of APPsα in the hippocampus of cDKO mice**

To investigate whether APPsα is able to rescue the synaptic deficits of NexCre cDKO (further referred to as cDKO) animals *in vivo*, APPsα was expressed in the adult brain using stereotactic injection. We employed an AAV9-based bicistronic vector (AAV-APPsα; Figure 1A) coding for Venus and codon-optimized HA-tagged murine HA-APPsα that was inserted behind the APP signal peptide (SP). The HA-APPsα reading frame was fused to Venus by a T2A site to enable tracking of transduced cells. Expression was driven by the neuronal synapsin promoter. Monocistronic AAV-Venus vector served as a control (AAV-Venus; Figure 1A). Adult cDKO animals (aged 4-5 months) were either injected with AAV-APPsα or AAV-Venus control vector, whereas littermate control mice (LM control) received only AAV-Venus. Thus, comparison of AAV-Venus injected cDKO mice with AAV-Venus injected LM controls was expected to yield similar synaptic impairments as previously observed for uninjected cDKO mice (Hick et al, 2015). Vectors were bilaterally injected (dose: $1.0 \times 10^9$ gc/µl per injection spot) into the stratum lacunosum moleculare region of the dorsal hippocampus and into the dentate gyrus (Figure 1B). To evaluate Venus and APPsα expression, animals were sacrificed 6 weeks post-injection and brain samples were analysed by Western blotting and immunohistochemistry using an HA-tag-specific antibody. Analysis of serial anteroposterior coronal brain sections (Bregma -1.10 to Bregma -2.70; see Figure EV1C) revealed widespread expression of APPsα in the hippocampus and, to a considerably lower extent, also in adjacent cortical areas (Figure 1C and EV1C). Along the longitudinal (dorsal-ventral) axis of the hippocampus APPsα expression was much more pronounced in the dorsal region, whereas expression was not detectable in the ventral hippocampus (Figure EV1C).

As Venus contained a membrane anchor, we observed prominent accumulation in dendritic regions, whereas HA-APPsα staining was intense in intracellular membrane compartments consistent with the transport of APPsα within the secretory pathway to the cell surface for secretion (see also Figure EV2D and E for co-localization with ER and Golgi markers). In the CA3 region APPsα expression appeared slightly lower compared to the expression obtained in pyramidal cells of the subiculum, the CA1 (Figure 1C) and the CA2 regions. Consistent with previous studies (Jackson et al, 2016), we noted that APPsα was detectable in HEK cells transfected with synapsin promoter driven AAV constructs (see Figure EV2A), likely due to AAV plasmid overload. *In vivo*, however, AAV-APPsα expression was restricted to neurons, as shown by double immunofluorescence staining against the HA-tag and the neuronal marker NeuN (Figure 1D and E). Consistently, no overlapping expression pattern was detectable in astrocytes (GFAP; Figure 1F) and microglia (Iba1; Figure 1G). The AAV-Venus expression pattern obtained from injections of control vector was largely similar to that

of AAV-APPsα (Figure EV1C). Western blot analysis of hippocampal homogenates, using the M3.2 antibody, confirmed efficient AAV-mediated APPsα expression in injected animals (Figure 1H). Note that antibody M3.2 is directed against an epitope located between the α- and β-secretase site and recognizes endogenous APP species (APP full length and APPsα) that are still expressed by interneurons and glia in cDKO mice (see Hick et al, 2015) and vector-derived AAV-APPsα. Collectively, our data demonstrate that AAV-APPsα injection results in efficient and neuron specific expression of APPsα throughout the dorsal hippocampus.

**Impaired synaptic plasticity and reduced spine density of APP/APLP2 cDKO mice are rescued by APPsα expression in the adult brain**

Having demonstrated that HA-APPsα is efficiently expressed in the hippocampus of injected animals we evaluated whether APPsα is sufficient to rescue impairments in functional network activity that were previously reported for cDKO mice (Hick et al, 2015). After 20 min of baseline recording we induced long-term potentiation (LTP) at the Schaffer collateral to CA1 pathway by application of theta burst stimulation (TBS) in acute hippocampal slices of mice that had been injected with viral vectors 6 weeks earlier (at 4-5 months of age). Consistent with our previous results (Hick et al, 2015) AAV-Venus injected cDKO mice exhibited significantly lower induction and maintenance of LTP (n=25 slices), as compared to AAV-Venus injected LM controls (n=23; Figure 2A). AAV-Venus injected LM control mice showed a potentiation of 156.69 ± 4.75% (t75-t80 after start of baseline recording) that was significantly reduced to only 128.12 ± 3.41% in AAV-Venus injected cDKO mice (one-way ANOVA followed by Bonferroni's *post-hoc* test, $^{###}p<0.001$; Figure 2B). In contrast, the LTP curve recorded from acute slices of AAV-APPsα injected cDKO mice (n=24) closely overlapped with that of AAV-Venus injected LM controls (Figure 2A). Quantification of the last five minutes of recording revealed that APPsα rescued LTP deficits of cDKO mice to a level statistically indistinguishable from that of Venus injected LM controls (t75–80: 150.34 ± 3.55%, $^{ns}p>0.05$; Figure 2B,). While basal synaptic transmission was comparable in all groups (see Figure EV3A and B), presynaptic function evaluated by paired pulse facilitation (PPF; Figure 2C) was significantly impaired in AAV-Venus injected cDKO mice at the 10 ms ($^{#}p<0.05$) and 20 ms ($^{#}p<0.05$) inter-stimulus intervals (ISI) in comparison to AAV-Venus injected LM controls. Strikingly, AAV-APPsα treatment lead to a highly significant rescue of short-term synaptic plasticity in cDKO mice as evidenced by PPF values statistically indistinguishable from LM controls (Figure 2C).

Next, we evaluated spine density as a correlate of excitatory synapses. To visualize neuronal morphology and spine density we performed iontophoretic postfixation filling with a

fluorescent dye of hippocampal CA1 pyramidal cells in brain slices from injected adult mice (aged 5-6 months). Consistent with our previous study (Hick et al, 2015), we confirmed a prominent reduction in spine density both in basal (100.00 ± 3.41 vs. 88.77 ± 3.10 spines/µm, $^{\#}p<0.05$) and in midapical dendrites (100.00 ± 3.57 vs. 82.08 ± 4.73 spines/µm, $^{\#}p<0.05$) of AAV-Venus injected cDKO CA1 neurons as compared to AAV-Venus injected LM controls (Figure 2D-F). In contrast, spine density of AAV-APPsα injected cDKO mice did not significantly differ from that of AAV-Venus injected LM control neurons, indicating that APPsα expression fully restored spine density in CA1 neurons. Collectively, these data demonstrate that acute expression of APPsα in adult mice is sufficient to rescue morphological and functional synaptic deficits in cDKO animals.

**APPsα ameliorates dendritic branching abnormalities of cDKO animals**

Prompted by the effect of APPsα on spine density we further evaluated its influence on the overall morphology and complexity of hippocampal CA1 neurons (Figure 3). In view of their different morphology and connectivity, basal and apical dendrites of CA1 neurons were studied separately. Neurons of AAV-Venus injected cDKO mice showed a significantly reduced total dendritic length and branching (as assessed by the number of nodes) in both basal and apical dendrites compared to AAV-Venus injected LM controls (Figure 3A-F). Impairments were readily apparent when visually inspecting reconstructed images of individual neurons (Figure 3A).

Although, total dendritic length was not significantly increased by APPsα expression in cDKO animals, the total number of nodes was significantly increased in basal dendrites of neurons from AAV-APPsα injected cDKO mice (Figure 3D) compared to injected LM controls (one-way ANOVA followed by Bonferroni's *post-hoc* test, $^{*}p<0.05$). To investigate whether changes in dendritic complexity may be more pronounced in distinct dendritic subregions we performed morphometric Sholl analysis. We plotted the dendritic length measured within circles centered on the soma against the distance from the cell body (see scheme in Figure 3B). In this analysis an increase in dendritic length per sphere corresponds to an increased dendritic complexity. Comparison of dendritic complexity between pyramidal neurons from AAV-Venus injected cDKO and LM control mice revealed an overall reduced dendritic complexity in cDKO neurons, both in basal (Figure 3G) and apical dendrites (Figure 3H). Branching deficits of basal dendrites from Venus injected cDKO neurons were most pronounced in proximal dendritic segments (60-90 µm from the soma). In apical dendrites both proximal regions (60-180 µm) and midapical regions (300 µm, 330 µm) were less complex. Interestingly, neurons of AAV-APPsα injected cDKO mice exhibited an intermediate curve with partially restored dendritic complexity. Sholl analysis revealed significantly increased dendritic complexity in proximal regions (60 µm) of basal dendrites of AAV-APPsα

injected cDKO neurons (repeated measures ANOVA followed by Sidaks's *post-hoc* test, *p<0.05; Figure 3G). Likewise, in apical dendrites of cDKO mice injected with AAV-APPsα, dendritic length was significantly increased at a distance of 300 µm (**p<0.01) and 330 µm (**p<0.01) as compared to neurons of AAV-Venus injected LM controls. Together, these data indicate that APPsα ameliorates branching deficits of cDKO mice at distinct dendritic regions.

**APPsα improves spatial learning and spatial memory in cDKO mice**

To evaluate whether improved synaptic density and plasticity is also reflected at the behavioural level we assessed spatial learning and memory in the Morris water maze (MWM) place navigation task (Figure 4). To this end, cDKO mice were either injected with AAV-Venus (n=18, red circles) or with AAV-APPsα (n=18, green circles) at 4-5 months of age and tested 2 months later at 6-7 months of age. For comparison, LM controls were injected with AAV-Venus (n=22, white circles). While swim speed was comparable in all groups (Figure 4A), we observed an overall impaired performance in AAV-Venus injected cDKO mice both during acquisition and reversal training. Although all three groups of mice did show learning, escape latencies (Figure 4B) and swim path lengths (Figure 4C) were significantly increased in Venus injected cDKO mice compared to Venus injected LM controls, especially at day 2 and 3 of acquisition, learning and for the last trials of reversal learning. These data are highly consistent with and confirm the deficit in spatial learning observed previously in uninjected cDKO mice (Hick et al, 2015). Overall, APPsα expression in cDKO mice lead to an intermediate performance with significantly improved learning during the reversal phase. AAV-APPsα expression in cDKO mice significantly decreased escape latency (**p<0.01; Figure 4B) and swim path length (*p<0.05; Figure 4C) at day 5 of reversal learning, as compared to AAV-Venus injected cDKO mice. During the probe trial that assesses spatial reference memory, analysis of time [%] spend in the target zone (Figure 4D) reveals a significant overall group x place effect ($F(2, 46)=9.095$, $p=0.0005$). AAV-Venus injected cDKO mice were strongly impaired in comparison to AAV-Venus injected controls ($^{###}p<0.001$) and failed to prefer the trained target zone. Time spend in the target zone was not significantly different from chance level. Strikingly, AAV-APPsα expression substantially improved probe trial performance in cDKO mice compared to Venus injected cDKO mice (**p<0.01). Moreover, AAV-APPsα injected cDKOs showed a highly significant preference for the trained target zone over adjacent quadrants (**p<0.01). Together these data indicate that expression of APPsα in the adult hippocampus substantially improved spatial reference memory.

**APPsβ does not rescue LTP or spine density *in vivo***

During AD BACE expression is upregulated (Ahmed et al, 2010; Holsinger et al, 2002) leading to increased amyloidogenic APP processing and thus increased APPsβ levels. Although APPsβ has been reported to be less potent in assays of neuroprotection *in vitro* (Barger & Harmon, 1997; Copanaki et al, 2010; Furukawa et al, 1996), the molecular basis of this property has remained elusive and its *in vivo* role is currently unknown. We therefore evaluated in a separate set of experiments whether APPsβ, being only 16 amino acids shorter compared to APPsα (see Figure 5B) might also be able to rescue the impairments of cDKO mice. Briefly, we stereotactically injected cDKO mice and LM controls (4-5 months of age) with AAV-Venus or a bicistronic vector coding for Venus and HA-tagged murine APPsβ (AAV-APPsβ; Figure 5A). Immunohistochemistry of serial brain sections indicated that injection of AAV-APPsβ yielded a comparable expression level and distribution to that obtained for HA-APPsα (Figure 5C and EV1C). Western blot analysis of total hippocampal homogenates (containing cell bound and secreted proteins), using an HA-tag specific antibody further confirmed similar (and statistically not significantly different) levels of HA-APPsß and HA-APPsα expression upon injection of the respective AAVs (unpaired two-tailed Student's t test, $^{ns}p>0.05$; Figure 5D and E). Ultracentrifugation (UC) of the same hippocampal homogenates was used to specifically detect soluble HA-APPsα and HA-APPsβ (Figure 5D, lower boxed panels) and yielded comparable levels of APPsα and APPsβ in UC supernatants (unpaired two-tailed Student's t test, $^{ns}p>0.05$; Figure 5D and E). Transfection of HEK cells (Figure EV2A) with the APPsα or APPsβ encoding AAV plasmids or transduction of cultured neurons with either of the AAV vectors (Figure EV2B), further confirmed efficient and comparable secretion of APPsα or APPsβ, as evidenced by Western blot analysis of cell supernatants (Figure EV2A and B).

Strikingly, despite comparable levels of expression and secretion, soluble APPsβ failed to exert any detectable effects on basal synaptic transmission (Figure EV3C and D) or synaptic plasticity in recordings of cDKO slices at 5-6 months of age (Figure 5F-H). AAV-APPsβ injected cDKO mice showed superimposable LTP curves compared to Venus injected cDKO mice. Potentiation at t75-80 min after start of baseline recording was not significantly different (cDKO + AAV-Venus: 125.58± 2.69% vs. cDKO + AAV-APPsβ: 124.42± 1.96%, $^{ns}p>0.05$, unpaired two-tailed Students t-test). Moreover, also in the PPF paradigm AAV-APPsβ expression did not lead to any detectable difference compared to AAV-Venus control injections (Figure 5H). Likewise, and consistent with electrophysiological analysis, AAV-APPsβ failed to rescue spine density deficits of cDKO mice determined upon Golgi staining. While AAV-Venus control-injected cDKO revealed again a significant reduction in spine density in basal and in apical dendrites as compared to Venus injected LM controls (Figure 5 I-K), spine density was comparable and statistically indistinguishable in AAV-Venus and

APPsβ injected cDKO mice. Basal dendrites from AAV-Venus and AAV-APPsβ injected cDKO mice showed a similar and statistically not significantly different reduction in spine density of 14% (LM control + Venus: 100 ± 2.84 vs. cDKO + APPsβ: 85.99 ± 3.51 spines/µm, *p<0.05) and 12% (LM control + Venus: 100 ± 2.84 vs. cDKO + APPsβ: 88.39 ± 3.26 spines/µm, ****p<0.0001), respectively (Figure 5J). In midapical dendritic segments spine density was reduced by 24% in AAV-Venus injected cDKO animals (LM control + Venus: 100 ± 4.01 vs. cDKO + APPsβ: 76.05 ± 2.097 spines/µm) or by 18% (LM control + Venus: 100 ± 4.01 vs. cDKO + APPsβ: 82.40 ± 3.28 spines/µm) in AAV-APPsβ injected animals, respectively (Figure 5K). Together, these data indicate a striking difference in the *in vivo* properties of secreted APPs, with APPsα ameliorating morphological and functional synaptic deficits of cDKO mice, while APPsβ had no effect.

**APPsα fascilitates LTP via its C-terminal CTα16 domain**

Prompted by these results, we now focused on the 16 amino acids domain (CTα16) that distinguishes APPsα from APPsβ (Figure 5B and L-Q). Previously, we had demonstrated that a short incubation of slices with recombinant APPsα (recAPPsα) is sufficient to largely rescue the LTP deficits of cDKO mice (Hick et al, 2015). To determine whether this acute function of APPsα is mediated by the CTα16 domain, we now pre-incubated cDKO slices for 60 min at room temperature either with a synthetic CTα16 peptide (10 nM), or with $CT\alpha16_{scr}$ (10 nM), a scrambled peptide of the same aminoacid composition. After 20 min of baseline recording LTP was again induced by TBS. During the whole measurement, the peptides diluted in ACSF were circulating in a closed-loop system. Strikingly, we found that the application of CTα16 potently facilitated LTP over the whole recording period (Figure 5L and M) including the early phase of post tetanic potentiation (t20-25, CTα16: 191.49 ± 8.82% vs. $CT\alpha16_{scr}$: 165.12 ± 8.82%, *p<0.01; Figure 5L and M) and led to a highly significant increase in LTP values 60 min after the beginning of baseline recording (t75-80, CTα16: 148.08± 3.65% vs $CT\alpha16_{scr}$: 132.18±4.26%, **p<0.01; Figure 5L and M). In contrast, LTP values obtained for $CT\alpha16_{scr}$ were very similar to those after AAV-Venus injection into cDKO mice (t75-80, 128.12 ± 3.41%; Figure 2B). CTα16 peptide application also significantly improved and restored PPF of cDKO slices (Figure 5N) to values comparable to that obtained for AAV-Venus injected LM control mice (see Figure 2C). Next, we directly compared the activity of CTα16 and recAPPsα (Figure 5O-Q) that was purified from stably transfected HEK cell supernatants. We found that CTα16 application (10 nM) was as potent as recAPPsα (10 nM) in rescuing both the LTP (t75-80, APPsα: 146.15 ± 4.21% vs. CTα16: 148.08 ± 3.65%, $^{ns}p>0.05$) and PPF deficit of cDKO slices. Values obtained were statistically indistinguishable (Figure 5O and P). Together, these data identify the CTα16 domain of APPsα as sufficient to mediate the functions of APPsα for enhancing synaptic plasticity *in vitro*.

**Inhibition of α7-nAChR by BTX blocks APPsα mediated effects on LTP**

While elevated (micromolar) Aβ concentrations and aggregated Aβ species exert an inhibitory effect on synaptic function (Gu & Yakel, 2011; Kamenetz et al, 2003; Shankar et al, 2007) picomolar concentrations of soluble Aβ have previously been demonstrated to enhance LTP and PTP in a mechanism dependent on the activity of α7 nicotinic acetylcholine receptors (α7-nAChRs; Puzzo et al, 2011; Puzzo et al, 2008). As the CTα16 domain of APPsα overlaps with the N-terminus of Aβ we therefore tested whether signalling by α7-nAChR may mediate the LTP fascilitating functions of APPsα. First, we hypothesized that if α7-nAChRs are crucially involved in the LTP facilitating effect of APPsα, pharmacological block of α7-nAChR should impair LTP in LM control mice that still express APP and APPsα. Indeed, treatment of slices with 10 nM α-bungarotoxin (α-BTX), a specific inhibitor of α7-nAChRs significantly reduced LTP during the induction phase (t20-25, LM control: 184.01 ± 5.59% vs. LM control + BTX: 158.04 ± 8.35%, *p< 0.05) and the maintenance phase (t75-80, LM control: 147.25 ± 3.87% vs. LM control + BTX: 131.24 ± 4.99%, *p<0.05; Figure 6A and B). Values obtained after BTX treatment were very similar to those of $CT\alpha16_{scr}$ treated cDKO slices (compare to Figure 5L and M, $CT\alpha16_{scr}$ treated cDKO slices: 132.18±4.26%).

Next, we tested how α-BTX affects the induction of LTP by exogenous application of recAPPsα or CTα16 (Figures 6,C-G and EV4). When α-BTX was added after the incubation of slices with either recAPPsα or CTα16 30 min before TBS (Figure EV4A, scheme) we observed only a small but not significant reduction in LTP (Figure EV4B-E). In contrast, if α-BTX was co-applied together with recAPPsα for 1 h before TBS (Figure 6C, scheme), this lead to a significant inhibition as compared to recAPPsα treatment alone (Figure 6D and E) both during the LTP induction phase (t20-25, recAPPsα: 168.49 ± 7.20% vs. recAPPsα + BTX: 142.57 ± 7.75%, *p<0.05; Figure 6D and E) and the LTP maintenance phase (t75-80, recAPPsα: 135.59 ± 3.81% vs recAPPsα: + BTX: 118.58 ± 5.51%, *p<0.05; Figure 6D and E). Similarly, if α-BTX was co-applied together with the CTα16 peptide for 1 h before TBS (Figure 6F and G) this lead to a significant inhibition as compared to CTα16 treatment alone both during the LTP induction phase (t20-25, CTα16: 176.23 ± 4.69% vs. CTα16 + BTX: 153.75 ± 5.05%, *p<0.05; Figure 6F and G) and the LTP maintenance phase (t75-80, CTα16: 144.37 ± 2.34% vs CTα16 + BTX: 129.24 ± 3.40%, **p<0.01; Figure 6F and G).Together, these data suggest that APPsα exerts its functions on LTP mainly via the CTα16 domain in a mechanism depending on α7-nAChR function. Further studies are needed to address whether improvements in spine density and behavior that we observed upon AAV-APPsα re-expression might also depend on α7-nAChR function.

**APPsα shows high-affinity allosteric potentiation of α7-nAChR expressed in *Xenopus* oocytes**

To investigate whether CTα16 can modulate α7-nAChRs directly, homomeric α7-nAChRs were expressed in *Xenopus laevis* oocytes, and nicotine- or acetylcholine-induced currents at submaximal $EC_{50}$ agonist concentration (100 µM) were measured either alone or after pre-application of CTα16 by two-electrode voltage clamp. Upon application of 100 nM of CTα16 alone, no activation of α7-nAChRs was detectable (data not shown). Remarkably, 100 nM of CTα16 significantly potentiated both, nicotine- and acetylcholine-induced currents with a 2.58 ± 0.42-fold (n=8) and 3.01 ± 0.96-fold (n=7) enhancement of α7-nAChRs responses, respectively (paired two-tailed Students t-test, *p<0.05; Figure 7A and B). In contrast, no significant effect of 100 nM $CT\alpha16_{scr}$ on nicotine-induced currents could be detected (1.00 ± 0.06-fold increase, paired two-tailed Students t-test, n=5; $^{ns}p>0.05$; Figure 7A and B). Dose-response analysis of CTα16 concentrations between 1 and 100 nM yielded an $EC_{50}$ value for CTα16 of 10.7 ± 4.6 nM with an hill-coefficient of 2.1 ± 0.3 for α7-nAChRs (n=4; Figure 7C). To establish if the physiological APPsα fragment that contains the CTα16 sequence at the C-terminus can also modulate α7-nAChRs, we tested the effect of 20 nM recAPPsα, a concentration close to the 10 nM that facilitated LTP in slices, on nicotine-gated currents (Figure 7D-F). Indeed, 20 nM recAPPsα significantly potentiated nicotine-induced currents (3.35 ± 0.81-fold enhancement, paired two-tailed Students t-test, n=4; *p<0.05) to a similar extent as seen with 100 nM of CTα16 (Figure 7D and E, see Figure 7A for comparison). This effect could be specifically blocked by BTX (Figure 7D). Notably, and consistent with experiments in slices, no significant potentiation was seen upon pre-application of 20 nM recAPPsβ (1.32 ± 0.18-fold enhancement, paired two-tailed Students t-test, n=5, $^{ns}p>0.05$; Figure 7D and E). As a further control, we investgated whether APPsα may also modulate other pentameric ion channels. However, neither recAPPsα nor CTα16 had any effects on the structuraly related homomeric α1-GlyR (Figure EV5), indicating that APPsα specifically acts on α7-nAChRs. Next, to gain further mechanistic insight, we analyzed nicotine dose-responses of α7-nAChRs in the presence of a potentiating concentration of recAPPsα to see how APPsα modulates α7-nAChRs (Figure 7F). Strikingly, 20 nM recAPPsα shifted the nicotine dose-response curve to the left and lead to an approximately 2-fold significant decrease in the $EC_{50}$ of nicotine (40.3 ± 6.0 µM) as compared to recAPPsα-free conditions ($EC_{50}$ of 80.5 ± 12.7 µM, paired two-tailed Students t-test, n=5, *p<0.05) without affecting the hill-coefficient (3.3 ± 0.5 vs. 3.7 ± 0.9, paired two-tailed Students t-test, $^{ns}p>0.05$) and the maximal inducible nicotine currents (Figure 7F). Together, these data indicate that APPsα

acts as a potent (nanomolar range) positive allosteric modulator of α7-nAChRs by increasing the apparent agonist affinity.

**Discussion**

In this study we provide compelling evidence for distinct functional roles of the two secreted APP ectodomain fragments APPsα and APPsβ *in vivo* and provide functional insight into the underlying mechanism. Long-term AAV mediated expression of either APPsα or APPsβ in the hippocampus of adult NexCre cDKO (cDKO) mice revealed several important new findings: (1) *In vivo* expression of APPsα efficiently rescued deficits in spine density, synaptic plasticity (LTP and PPF) and spatial reference memory of cDKO mice. (2) In contrast to APPsα, APPsβ expression had no detectable effects on spine density and synaptic plasticity in cDKO mice indicating distinct functional roles in the adult CNS. (3) The C-terminal 16 amino acids (CTα16) of APPsα proved sufficient to enhance LTP to the same extent as full length APPsα. (4) Nanomolar concentrations of APPsα and CTα16 function as potent positive allosteric modulators of α7-nAChRs *in vitro* increasing the apparent agonist affinity. (5) Pharmacological blockade of α7-nAChRs impaired the APPsα (or CTα16) induced LTP facilitation, thus identifying α7-nAChRs as physiological APPsα receptors that are involved to mediate its LTP enhancing effects.

Our previous studies indicated that LTP deficits of cDKO mice can be rescued by acute application of nanomolar amounts of APPsα, but not APPsβ, *in vitro*. Despite this, it remained unclear whether these acute *in vitro* effects observed for a short time of recording would also be relevant *in vivo* and whether reduced spine density and behavioural deficits might also be rescued. Lastly, the mechanism by which APPsα regulates LTP including the relevant receptor was unknown. In principle, synaptic deficits of cDKO mice may either arise from developmental deficits such as impaired synaptogenesis that could lead to miswired circuits or may be due to a lack of APP (or APLP2) mediated functions in the adult brain. To address these questions and to assess the specific role(s) of the secreted ectodomains APPsα and APPsβ we used a reverse genetic approach employing AAV mediated re-expression of APPsα or APPsβ in the hippocampus of adult cDKO mice. Expression of the AAV-Venus control vector in cDKO mice or littermate controls fully reproduced and confirmed the phenotype of cDKO mice (Hick et al, 2015) including reduced dendritic complexity (Figure 3) and spine density of CA1 neurons (Figure 2), deficits in synaptic plasticity (Figure 2), as well as impaired spatial memory (Figure 4). This indicates that the cDKO phenotype is robust and that AAV vectors are suitable to confer neuron-specific, long lasting and widespread expression throughout the dorsal hippocampus (see Figure 1 and EV1). Using this *in vivo* reconstitution approach we show that APPsα is not only able to acutely modulate synaptic

strength when applied *in vitro* (Hick et al, 2015), but has the ability to mediate a long-lasting rescue of synaptic plasticity, with facilitating effects on both the induction and maintenance phase of LTP. Moreover, we show that APPsα re-expression rescued spine density, a correlate of excitatory synapses. These data are perfectly in line with our recent observation that a lack of APP and thus APPsα in APP-KO mice leads to impairments in structural spine plasticity in the cortex (Zou et al, 2016). While synaptic plasticity and spine density were completely restored, APPsα expression led only to a partial rescue of dendritic complexity of CA1 neurons with effects most pronounced close to the soma and in the midapical region of apical dendrites. These more subtle effects might in part be related to a potential non-uniform secretion of APPsα along dendritic regions of individual neurons that may become limiting at more distant regions. Alternatively, it is conceivable that APPsα is not sufficient to fully restore neurite outgrowth and dendritic arbours due to an inability to compensate early developmental defects or an additional requirement for APLP2 (Hick et al, 2015; Weyer et al, 2014). In this regard neither deficiency of APP nor APLP2 alone results in reduced dendritic complexity in young adult knockout mice (Lee et al, 2010; Midthune et al, 2012; Tyan et al, 2012) while a combined absence in cDKO mice profoundly impairs neuronal morphology and total neurite length (Hick et al, 2015; Weyer et al, 2014 and this study) indicating a combined role of APP and APLP2 for dendritic complexity. Although the underlying mechanism is still unknown, it appears likely that domains conserved between APP and APLP2 (and not divergent sequences as those close to the secretase sites) are involved.

The ability of synapses to undergo long-term potentiation is considered as a cellular mechanism underlying learning and memory (reviewed by Korte & Schmitz, 2016). Indeed, restoration of LTP by APPsα at the CA3/CA1 pathway was associated with a significantly improved performance in water maze place navigation during reversal learning (Figure 4B and C) and a substantial increase of spatial reference memory, as assessed during the probe trial. In mice, emotional reactions to the swim stress are a limiting factor for performance during task acquisition, that becomes less relevant during reversal learning as mice get habituated to the stressfull experience (Lipp & Wolfer, 1998; Strange et al, 2014). The dorsal hippocampus known to mediate spatial processing (Strange et al, 2014) was efficiently targeted by AAV-APPsα injections whereas AAV-APPsα was undetectable in ventral portions of the hippocampus (Figure EV1C) that mediate emotional responses. Thus, a failure to compensate emotional problems due to selective targeting may explain why AAV-APPsα treatment effects were only seen during probe trial and reversal learning. Although hippocampal lesions have been demonstrated to impair hippocampus dependent behaviour (Morris et al, 1982), learning and memory also involves other brain regions that were not targeted by the injections including the dorsomedial striatum – prefrontal cortex network

which is strongly involved in the initial acquisition of the place-navigation task (Woolley et al, 2013). The entorhinal cortex constitutes a crucial interface between the hippocampus and the rest of the cortex and is the source of most spatial information that is processed in the hippocampus (Hales et al, 2014; Vorhees & Williams, 2014). The fact that AAV-APPsα expression was largely restricted to the hippocampus may thus underlie the partial but not complete rescue of performance by APPsα in the MWM. In addition, part of the behavioural deficits may also be due to developmental effects of the mutation.

Consistent with an important function of APPsα in the mature brain, acute depletion of APPsα in wild type animals by either pharmacological inhibition of α-secretase (Taylor et al, 2008), or infusion of antibodies directed against the C-terminus of APPsα (Puzzo et al, 2011; Taylor et al, 2008) was reported to impair LTP and cognitive behaviour. However, these previous approaches lacked specificity, as secretase inhibitors target also other substrates besides APP and antibodies employed bind not only to APPsα, but also full length APP and Aβ. Collectively, our findings indicate an important synaptotrophic role of APPsα in the mature CNS for spine density, synaptic plasticity and behaviour. Importantly, this role in cDKO mice (lacking endogenous APP and all its fragments including Aβ) is completely independent of any indirect Aβ related effects, in contrast to the previously reported Aβ lowering properties of APPsα in transgenic APP/PS1 mice (Fol et al, 2016; Obregon et al, 2012).

As APPsα and APPsβ are identical in primary sequence except for the last 16 amino acids one may expect that they should exhibit largely overlapping functions. Accordingly, only few studies have so far directly compared the effects mediated by either APPsα or APPsβ. *In vitro*, recombinant APPsβ was shown to more efficiently induce the differentiation of human embryonic stem cells into neuronal precursor cells as compared to APPsα (Freude et al, 2011), whereas a similar increase in axonal outgrowth was observed when cortical neurons were treated with recombinant APPsβ or APPsα (Chasseigneaux et al, 2011). With regard to *in vitro* assays of neuroprotection, however, APPsβ was considerably less potent to protect neurons against glucose deprivation, excitotoxicity, Aβ peptide (Barger & Harmon, 1997; Furukawa et al, 1996) or epoxomycin induced proteasomal stress (Copanaki et al, 2010).
Here, we compared the *in vivo* functions of the secreted APP ectodomains in the adult brain. A surprising key finding was that APPsα and APPsβ exert strikingly different effects *in vivo*, despite a similar level and pattern of AAV mediated expression (Figure 1, 5C-E, EV1 and EV2). Of note, the absence of beneficial effects by AAV-APPsβ cannot be simply explained by possible adverse effects associated with AAV-APPsβ expression, as we found no signs of increased cell death, nor any evidence for increased astrogliosis or microgliosis (see

Appendix Figure S1) as unspecific signs of neurodegeneration. Although earlier studies had indicated that a fragment (N-APPs1-286) derived for APPsβ may induce caspase activation and death of sensory neurons in a mechanism requiring DR6 (Nikolaev et al, 2009), these data have recently been revised to exclude a requirement for β-secretase activity and thus APPsβ (Olsen et al, 2014). Our notion that AAV-APPsβ does not induce adverse effects is further in agreement with previous data from fully viable and apparently normal APPsβ-knockin (KI) mice expressing solely APPsβ (Li et al, 2010). However, whether a lack of APPsα in APPsβ-KI mice might lead to synaptic deficits, had remained unknown.

We demonstrate that APPsα but not APPsβ rescues spine density and synaptic plasticity in cDKO mice and identified the CTα16 peptide of APPsα as a crucial LTP-enhancing functional domain that stimulated LTP to the same extent as recAPPsα. Mechanistically, facilitation of LTP by APPsα or the CTα16 peptide required functional α7-nAChRs since BTX, a selective antagonist of α7-nAChRs, blocked its effects. Moreover, when expressed in oocytes α7-nAChRs could be directly potentiated by nanomolar amounts of either recAPPsα (but not recAPPsβ) or CTα16 in the presence of ACh or nicotine. Together with the ability of APPsα to enhance the sensitivity of α7-nAChRs for the agonist nicotine these data establish APPsα as a potent positive allosteric modulator (PAM). These findings also suggests that APPsα might function as an endogenous PAM of cholinergic signalling in the hippocampus, although further studies are required to confirm its activity *in vivo*. Several studies have shown that nAChR activation facilitates LTP induction (for review see Yakel, 2014) and α7-nAChRs are abundantly expressed in the hippocampus (Fabian-Fine et al, 2001). Several mechanisms of α7-nAChR signalling are thought to contribute to its role in LTP: (1) activation of presynaptic α7-nAChR can increase glutamate release and (2) activation of postsynaptic α7-nAChRs that have a high $Ca^{2+}$ permeability may confer membrane depolarisation and induce plasticity evoking $Ca^{2+}$ signalling (reviewed by McKay et al, 2007; Yakel, 2014).

Although more work is needed to understand precisely how APPsα affects α7-nAChR signalling, we hypothesize that both pre- and postsynaptic mechanisms are likely to play a role. When applied to slices of cDKO mice both APPsα and CTα16 enhanced, in a BTX sensitive manner, the early phase of post tetanic potentiation (PTP) during the first minutes after start of baseline recording, which is believed to involve $Ca^{2+}$ build up in presynaptic terminals. In line with an important endogenous role of APPsα for LTP, APPsα secretion is activity dependent (Fazeli et al, 1994; Gakhar-Koppole et al, 2008; Hoey et al, 2009; Mills & Reiner, 1999) and can thus be increased through plasticity-inducing protocols. The enhancement of PTP by APPsα, as observed in our study, is consistent with previous studies that reported increased $Ca^{2+}$ influx in synaptoneurosomes and α7-nAChRs transfected

neuroblastoma cells upon application of low (picomolar) amounts of Aβ or N-terminal Aβ-peptides that are overlapping with the C-terminus of APPsα (Dougherty et al, 2003; Lawrence et al, 2014). Recently, low (picomolar) amounts of Aβ were shown to increase synaptic vesicle recycling via α7-nAChRs signalling in hippocampal neurons (Lazarevic et al, 2017). Moreover, low amounts of Aβ had been found to stimulate synaptic plasticity and memory in wildtype mice (Lawrence et al, 2014; Puzzo et al, 2011). In addition to presynaptic effects it is likely that APPsα will also modulate signalling at the postsynaptic site (for a review see Ludewig & Korte, 2016). Postsynaptic membrane depolarization due to $Ca^{2+}$ influx through α7-nAChRs may increase the probability of NMDAR-activation that is crucial to induce stable LTP. In this regard, NMDAR inhibition has been shown to prevent the nicotine induced conversion of short term plasticity into LTP at CA3/CA1 synapses (Ji et al, 2001). Moreover, also activation of septal cholinergic input to induce α7-nAChRs dependent LTP required the activation of NMDARs (Gu & Yakel, 2011). In addition to signalling via α7-nAChRs, APPsα may also modulate NMDARs more directly, as APPsα was shown to potentiate tetanically evoked NMDAR currents recorded at granule cells of the dentate gyrus in rat brain slices (Taylor et al, 2008) and full length APP can biochemically interact with GluN1/GluN2 receptors (Cousins et al, 2009), although it is not known whether this also holds true for APPsα.

Our finding of distinct *in vivo* functions of APPsα and APPsβ have important implications for AD therapy. Pharmacological BACE1 inhibition has been a major approach to reduce Aβ in animals and clinical trials. More recently, however, concerns have been raised that therapeutic BACE1 inhibition may also compromise important physiological functions of BACE (Yan & Vassar, 2014). Indeed, BACE-KO mice display deficits in synaptic morphology and synaptic plasticity in the hippocampus (Dominguez et al, 2005; Laird et al, 2005). Our data indicate that APPsβ deficiency that will result from BACE1 inhibition is unlikely to lead to toxic effects. A number of recent studies demonstrated that very low concentrations of Aβ may stimulate transmitter release and modulate synaptic plasticity and memory. Strikingly, we now show that it is the C-terminal domain of APPsα that is required to increase spine density, rescues LTP and improves memory. As this C-terminal domain is also present in Aβ it is conceivable that signaling of APPsα and soluble, non-aggregated Aβ species may at least in part converge e.g. regarding the activation of α7-nAChRs. From this, one may speculate that APPsα may be able to rescue potential unwanted effects due to limited Aβ levels. It is also clear however, that sequence context and structural accessibility of the CTα16 domain is crucial. Indeed, the presence of the CTα16 domain appears to alter the conformation of APPsα in a critical way. Analysis by small angle X-ray diffraction indicated that the 3D structure of APPsα and APPsβ are quite different, with APPsα exhibiting an extended conformation with an exposed CTα16 region (Gralle et al, 2006; Peters-Libeu et al,

2015) while in APPsβ the E1 domain folds back towards the C-terminal juxtamembrane region (Peters-Libeu et al, 2015). Thus, different 3D structures may enable or prevent binding to receptors, which may contribute to the distinct *in vivo* functions of APPsα and APPsβ.

Regarding AD pathogenesis our data indicate that a shift towards β-secretase processing that may occur during AD (Ahmed et al, 2010; Holsinger et al, 2002) could result in insufficient amounts of APPsα that cannot be functionally compensated by APPsβ. A lack or reduction of APPsα might thus contribute to deficits in synaptic plasticity and cognitive function in AD patients. Consistent with this notion, in AD patients hippocampal levels of synaptically localized ADAM10/SAP97 complexes are reduced (Epis et al, 2010) and an activity attenuating mutation in the human ADAM10 gene leading to reduced APPsα levels has been associated with AD (Kim et al, 2009; Suh et al, 2013). In familial AD cases carrying the Swedish mutation ($APP_{SWE}$), cerebrospinal fluid (CSF) levels of APPsα were found to be reduced already at early stages of AD (Almkvist et al, 1997; Lannfelt et al, 1995). With regard to sporadic AD cases, however, no consensus has been reached whether reduced APPsα levels are associated with AD (Mockett et al, 2017). Early on, total soluble APPs was reported to be severely reduced in sporadic AD (Van Nostrand et al, 1992). Several more recent studies specifically detecting APPsα reported reduced APPsα levels in mild (Sennvik et al, 2000) to moderate AD (Rosen et al, 2012), or in APOE4 positive AD patients (Olsson et al, 2003), while several other studies found no difference in APPsα at early stages of sporadic AD (Brinkmalm et al, 2013; Perneczky et al, 2013; Perneczky et al, 2011; Rosen et al, 2012). These mixed results may in part be related to different assays systems used and the recent finding of considerable diurnal variability of APPsα and other APP fragments in the CSF (Dobrowolska et al, 2014). Thus, more detailed studies are needed to resolve these important issues.

In preclinical AD research, numerous studies have been conducted using APP transgenic mouse models (https://www.alzforum.org/research-models) which (over)express $huAPP_{SWE}$ that undergoes enhanced β-secretase processing. Consequently, reduced levels of neuroprotective and synaptotrophic APPsα relative to increased amounts of Aβ (that is a reduced APPsα/Aβ ratio) may fail to adequately balance and antagonize the detrimental effects of Aβ and thus contribute to the phenotypic and synaptic impairments in these transgenic mice.

APPsα also plays an important role in processes of natural aging. Not only is memory performance correlated with APPsα levels in rats (Anderson et al, 1999), but also aging-

related deficits in LTP and cognitive behaviour can be rescued by exogenous APPsα (Moreno et al, 2015; Xiong et al, 2016). Collectively, our findings indicate distinct functions for APPsα in the adult brain to modulate spine density, synaptic plasticity and cognition. Our findings lend further support to therapeutic approaches aimed at directly or indirectly increasing APPsα expression in the brain (Mocket et al., 2017, Müller et al, 2017) to ameliorate synaptic deficits in AD or possibly also for other diseases in which neurons are damaged.

**Author contributions**

UCM designed and conceived the study. MCR, SK and SW performed intracranial AAV injections and analyzed data. CB and KH produced recombinant APPs. MCR performed and analyzed experiments related to APPs expression analysis in vitro and in vivo including Western blots, performed immunochemistry and Sholl analysis including spine density measurements. SL, LRS and MK performed extracellular electrophysiological recordings in brain slices, analyzed data and interpreted results. AW and BL performed electrophysiological recordings in oocytes, analyzed data and interpreted results. TA and CJB generated AAV vectors and analyzed APPs expression in neurons. AKF and DPW conducted, analyzed and interpreted behavioral experiments. UCM wrote the manuscript with help from MCR and input from all authors.

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## Figure Legends

**Figure 1**: **Expression of APPsα in the hippocampus of AAV-APPsα injected cDKO animals. A** Schematic representation of monocistronic and bicistronic AAV constructs enabling neuron-specific expression of i) Venus and ii) HA-tagged APPsα (+ Venus). ITR: inverted terminal repeat, Synapsin: promotor, T2A: *Thoseaasigna* virus 2A site, SP: signal peptide, HA: influenza hemagglutinin tag. **B** Scheme of the hippocampus with coordinates of the two injection sites (black stars). **C** Overview of the hippocampus of an AAV-APPsα injected cDKO mouse. HA-tag staining (red) reveals APPsα expression within the CA1, CA3 and DG of the hippocampus. Magnification shows the boxed CA1 region. Scale bars: 500 µm (left), 100 µm (right). DG: Dentate Gyrus, CA: Cornus Ammonis. **D-G** Double immunostaining in CA1 region. APPsα (HA-tag, red) is exclusively expressed in neurons (NeuN, blue, **D, E**), but not in astrocytes (GFAP, blue, **F**) or microglia (Iba1, blue, **G**). Scale bars: 10 µm (**D, F, G**), 5 µm (**E**). **H** Western blot analysis of APP expression in hippocampus of LM control (N=5) and cDKO mice (N=5) injected with AAV-Venus or AAV-APPsα. Age of mice: 5-6 months.

**Figure 2: AAV-APPsα rescues LTP, impaired short-term synaptic plasticity and spine density of cDKO mice**. **A, B** LTP was induced at hippocampal CA3-CA1 synapses after 20 min baseline recordings (arrowhead, TBS). cDKO mice expressing Venus (red) exhibited significant lower induction and maintenance of LTP (128.12 ± 3.41%) compared to Venus injected LM controls (white, 156.69 ± 4.75%, $^{###}p<0.001$). AAV mediated expression of APPsα (green) restored potentiation after start of baseline recording and resulted in an LTP curve comparable to that of LM controls. The LTP induction rate is shown as percentage % of mean baseline slope. Data points were averaged over 6 time points. **C** The deficit of PPF in Venus injected cDKO mice at the 10 ms ($^{#}p<0.05$) and 20 ms ($^{#}p<0.05$) ISI was restored by expression of APPsα. n=number of slices. N=number of animals. **D** Representative images of basal and midapical dendritic segments of CA1 neurons. **E, F** The spine density deficit of Venus injected cDKOs mice (11% in basal and 18% in midapical dendrites) is rescued by APPsα to LM control levels. Images are maximum projections of deconvolved *z*-stacks. Scale bar: 5 µm. Spine density was normalized to LM control levels. n=number of neurons (from 5 animals per condition). **A-F** Age of mice at analysis: 5-6 months. Data represent mean ± SEM. Data were analyzed by one-way ANOVA followed by Bonferroni´s *post-hoc* test. # indicates significant differences between LM control and cDKO injected with AAV-Venus, * between cDKO injected with AAV-Venus or APPsα. $^{ns}p>0.05$, $^{*/\#}p<0.05$, $^{**/\#\#}p<0.01$, $^{***/\#\#\#}p<0.001$.

**Figure 3: APPsα ameliorates dendritic branching abnormalities of cDKO animals**

**A** Representative 3D-reconstructions of CA1 pyramidal neurons from AAV-Venus injected LM controls (left), AAV-Venus injected cDKOs (middle, red) and AAV-APPsα injected cDKOs (right, green). **B** Schematic representation of parameters assessed. **C-F** Compared to LM controls AAV-Venus injected cDKO mice show a significantly reduced basal ($^{\#\#}p<0.01$, **C**) and apical ($^{\#\#}p<0.01$, **E**) dendritic length and reduced branching in basal ($^{\#\#}p<0.01$, **D**) and apical ($^{\#\#}p<0.01$, **F**) dendrites. AAV-APPsα injection did neither affect basal ($^{ns}p>0.05$, **C**) nor apical ($^{ns}p>0.05$, **E**) total dendritic length. However, the total number of basal nodes differed significantly compared to AAV-Venus injected cDKO mice (*p<0.05, **D**). **G** Sholl analysis of basal dendritic length reveals a significant group effect (repeated measures ANOVA: genotype F(2,

29)=5.038, p<0.05) and a significant distance effect (repeated measures ANOVA: genotype F(5, 145)=250.2, p<0.0001). Due to a significant interaction effect (repeated measures ANOVA: genotype F(10, 145)=4.466, p<0.0001), a *post-hoc* Sidak's multiple comparison test was performed to further evaluate effects between groups at distinct distances from soma. Compared to Venus injection, AAV-APPsα injection of cDKO significantly increased basal dendritic length at 60 µm (*p<0.05). **H** Sholl analysis of apical dendritic length reveals an overall significant group effect (repeated measures ANOVA: genotype F(2, 37)=4.776, p<0.05) and a significant distance effect (repeated measures ANOVA: genotype F(18, 666)=47.54, p<0.0001). Due to a significant interaction effect (repeated measures ANOVA: genotype F(36, 666)=1.640, p<0.05), a *post-hoc* Sidak's multiple comparison test was performed to further evaluate effects between groups at distinct distances from soma. AAV-APPsα expression increased midapical dendritic length at 300 µm (**p<0.01) and 330 µm (**p<0.01) distance from soma compared to AAV-Venus injected cDKO mice. **A-H** Age of mice: 5-6 months. n=number of neurons (from 4-5 animals per condition). Data represent mean ± SEM. Data were analyzed by one-way ANOVA followed by Bonferroni´s *post-hoc* test (**C-F**) or repeated measures ANOVA followed by Bonferroni´s or Sidak's *post-hoc* test (**G-H**). # indicates significant differences between LM control and cDKO injected with AVV-Venus, * between cDKO injected with AAV-Venus or APPsα, ● between LM control injected with Venus and cDKO injected with AAV-APPsα. $^{ns}$p>0.05, $^{*/\#/\bullet}$p<0.05, $^{**/\#\#/\bullet\bullet}$p<0.01, $^{***/\#\#\#/\bullet\bullet\bullet}$p<0.001.

**Figure 4: APPsα improves place navigation and spatial memory of cDKO mice in the MWM A** Swim speed during acquisition and reversal learning is comparable in all groups (each point represents one day with 6 trials). **B** During acquisition learning and after platform reversal to the opposite quadrant, AAV-Venus injected cDKOs (red circles) show considerably longer escape latency compared to AAV-Venus injected LM controls (white circles). AAV-APPsα injected cDKO mice (green circles) show an overall intermediate performance (genotype F(2, 46)=6.361, p=0.0036) and that differs significantly at day 5 of reversal learning compared to AAV-Venus injected cDKO mice (genotype x day F(8,184)=5.266, p<0.0001; **p<0.01). **C** Measurements of swim path length confirms the training performance deficit of cDKO mice compared to LM controls. During the reversal phase, the performance of AAV-APPsα injected cDKOs is significantly improved at day 5 (genotype F(2,46)=16.70,

p<0.0001, genotype x day F(8,184)=5.788, p<0.0001; *p<0.05, ••p<0.01). **D** In the probe trial, AAV-Venus injected cDKOs (red) spend significantly less time in the target zone compared to AAV-Venus ($^{###}$p<0.001) injected LM control animals (white) and failed to prefer it significantly over control zones in adjacent quadrants. Note that expression of APPsα (green) largely rescues the deficits of cDKOs. N=22 (LM controls: AAV-Venus), N=18 (cDKOs: AAV-Venus), N=18 (cDKOs: AAV-APPsα). Data represent mean ± SEM. Dashed lines: chance level. Data were analyzed using a mixed ANOVA model with conditions (LM control: AAV-Venus, cDKO: AAV-Venus and cDKO: AAV-APPsα) as between subject factor. Within subject factors were added to explore the dependence of genotype effects on day and place. Significant interactions and main effects were further explored by pairwise FDR-corrected two-tailed Student's t-tests. # indicates significant differences between LM control and cDKO injected with AVV-Venus, * between cDKO injected with AAV-Venus or APPsα, ● between LM control injected with Venus and cDKO injected with AAV-APPsα $^{ns}$p>0.05, $^{*/\#/\bullet}$p<0.05, $^{**/\#\#/\bullet\bullet}$p<0.01, $^{***/\#\#\#/\bullet\bullet\bullet}$p<0.001.

**Figure 5**: **AAV-APPsβ fails to rescue LTP or spine density *in vivo*, while CTα16 facilitates LTP *in vitro*. A** Schematic representation of AAV9 constructs enabling the neuron-specific expression of i) Venus and ii) HA-tagged APPsβ (+ Venus). ITR: inverted terminal repeat, Synapsin: neuron specific promotor, T2A: *Thoseaasigna* virus 2A site, SP: signal peptide, HA: influenza hemagglutinin tag. **B** APPsα differs from APPsβ in the last 16 C-terminal amino acids (CTα16, green). The epitope recognized by the M3.2 antibody is indicated. **C** Overview of the hippocampus of a AAV-APPsβ (HA-tag, red) injected cDKO mouse. APPsβ is expressed in CA1, CA3 and DG. Scale bar: 500 µm. DG: dentate gyrus, CA: Cornus Ammonis. **D** Western blot analysis of HA-APPsα and HA-APPsβ in hippocampi of injected mice (boxed upper panel), probed with an HA-tag specific antibody (lower band, arrowhead; *unspecific signal). Homogenates of AAV injected hippocampi were subjected to ultracentrifigation to detect soluble APPsα and APPsβ (UC supernatant; boxed lower panel). The APP C-terminal antibody Y188 was used to confirm the separation of soluble APPsα from membrane bound full-length APP that is detected in total lysates of AAV-Venus injected littermate controls. Hippocampi of $APP^{-/-}$ mice served as a negative control for antibody specificity. **E** Quantification of HA-APPsα and HA-APPsβ expression in total hippocampal homogenates and of soluble HA-APPs after

UC normalized to β-tubulin. Note the comparable expression in AAV-APPsα and AAV-APPsβ injected mice. Age: 5-6 months. N=Number of animals. Data represent mean ± SEM. Data were analyzed by unpaired two-tailed Student's t test. $^{ns}p>0.05$. **F-H** Activity dependent synaptic plasticity was investigated in cDKO after injection of AAV-Venus (red) or APPsβ (blue). **F, G** AAV-mediated overexpression of APPsß (n=23 slices, blue symbols) failed to rescue the LTP defect of cDKO mice (n=24 slices, red symbols). **G** TBS induced strengthening of fEPSPs resulted in similar potentiation levels for the last 5 minutes of recording (t75-80). The LTP induction rate is shown as percentage % of mean baseline slope. Data points were averaged over 6 time points. **H** PPF paradigm yielded no significant differences between viral vector injected cDKO mice. Age: 5-6 months. n=number of recorded slices. N=number of animals. Data represent mean ± SEM. Data were analyzed unpaired two-tailed Students t-test. **I** Representative images of Golgi stained midapical and basal dendritic segments of LM control and cDKO animals injected with AAV-Venus or AAV-APPsβ. **J, K** The significant spine density deficit of AAV-Venus injected cDKO mice (red bar) in basal (**J**) and apical dendrites (**K**) was comparable and not significantly different ($^{ns}p>0.05$) from that of AAV-APPsβ injected cDKO mice (blue bar). White bar: AVV-Venus injected LM controls. Images are maximum projections of z-stacks. Scale bar: 5 µm. Spine density is normalized to LM control levels. Age: 5-6 months; n=number of neurons (from 5 animals per condition). Data represent mean ± SEM. Data were analyzed by one-way ANOVA followed by Bonferroni´s *post-hoc* test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 . **L, M** Acute hippocampal slices of cDKO were pre-incubated for 1 hour either with 10 nM CTα16 or $CT\alpha16_{scr}$ before inducing LTP by TBS (arrowhead). Averaged potentiation levels at LTP induction (t20-25) revealed a significantly enhancement by CTα16 as compared to $CT\alpha16_{scr}$ present over the whole recoding time. Potentiation levels at t75-80 are significantly increased in the presence of CTα16 (**p<0.01). The LTP induction rate is shown as percentage % of mean baseline slope. Data points were averaged over 6 time points. **N** CTα16 significantly enhances short-term synaptic plasticity at inter-stimulus intervals of 40 (**p<0.01), 80 (*p<0.05), and 160 (**p<0.01) ms compared to $CT\alpha16_{scr}$. **O-Q** Acute application of CTα16 and recAPPsα elevates LTP in cDKO slices to the same extent. **O, P** After 1 hour pre-incubation with either 10 nM CTα16 or 10 nM recAPPsα fEPSPs were recorded. The TBS induced LTP curve was closely overlapping for both conditions and resulted in similar, statistically not significantly

different LTP values **Q** CTα16 and recAPPsα modulate presynaptic function of cDKO mice in the same manner. Age: 5-6 months. n=number of recorded slices. N=number of animals. Data represent mean ± SEM. Data were analyzed by unpaired two-tailed Students t-test.

**Figure 6: LTP facilitation by APPsα or CTα16 requires functional α7-nAChRs**

**A, B** Treatment with 10 nM α-BTX (30 min prior to TBS, black circles) significantly reduces LTP induction (t20-25, phase of post tetanic potentiation) and maintenance (t75-80) in LM controls expressing endogenous APP and APPsα. The LTP induction rate is shown as percentage % of mean baseline slope. Data points were averaged over 6 time points. **C** Experimental set-up: co-application of recAPPsα or CTα16 and BTX before LTP induction. Peptides and BTX inhibitor used for pre-incubation circulated throughout the whole LTP recording. **D, E** Co-application of BTX and recAPPsα significantly inhibits synaptic plasticity in cDKO mice. After 1 hour pre-incubation of acute slices with 10 nM recAPPsα or 10 nM recAPPsα and 10 nM BTX fEPSPs were recorded. Hippocampal acute slices treated with 10 nM BTX and recAPPsα revealed significantly impaired induction (t20-25) and maintenance (t75-80) of LTP in comparison to slices of cDKO mice recorded in the presence of recAPPsα alone. **F, G** Co-application of BTX and CTα16, the C-terminal domain of APPsα, significantly inhibits synaptic plasticity in cDKO mice. After 1 hour pre-incubation of acute slices with 10 nM CTα16, or 10 nM CTα16 and 10 nM BTX fEPSPs were recorded. Hippocampal acute slices treated with 10 nM BTX and CTα16 revealed similar to recAPPsα, a significantly impaired induction (t20-25) and maintenance (t75-80) of LTP in comparison to slices of cDKO mice recorded in the presence of CTα16 alone. Age: 5-6 months of age. n=number of recorded slices. N=number of animals. Data represent mean ± SEM. Data were analysed by unpaired two-tailed Students t-test. *p<0.05, **p<0.01.

**Figure 7: Potentiating effect of CTα16 and APPsα on recombinant homomeric α7-nAChRs. A** Nicotine- and acetylcholine-induced whole-cell current traces of α7-nAChRs expressed in *Xenopus* oocytes recorded in the absence or presence of CTα16 or $CT\alpha16_{scr,}$ respectively. Note specific robust potentiation of nicotine- and acetylcholine-induced currents by CTα16, but no direct activation of α7-nAChRs by either peptide in the absence of agonist (not shown). Bars indicate application of

$EC_{50}$ agonist concentrations and of the peptide indicated (green: CT$\alpha$16: grey: CT$\alpha16_{scr}$). **B** Quantification of potentiation shows that CT$\alpha$16 enhances nicotine- and acetylcholine-induced currents to a similar extend whereas CT$\alpha16_{scr}$ had no effect. **C** CT$\alpha$16 dose-response curve obtained with submaximal nicotine concentrations (100 µM) with a corresponding $EC_{50}$ value of 10.7 ± 4.6 nM and a hill-coefficient of 2.1 ± 0.3 (n=4). **D** Nicotine-induced whole-cell current traces of $\alpha$7-nAChRs in the presence of 20 nM recAPPs$\alpha$ (green bar) or 20 nM recAPPs$\beta$ (blue bar). Note that only recAPPs$\alpha$ efficiently potentiates $\alpha$7-nAChRs, which is blocked by BTX (orange bar). **E** Quantification of potentiation shows that recAPPs$\alpha$ enhances nicotine-induced currents to a similar extend as CT$\alpha$16 (see for comparison Figure 7B) while recAPPs$\beta$ had no effect. **F** Nicotine dose-response curve obtained either in the presence of 20 nM recAPPs$\alpha$ (green circles, left) or without treatment (black circles, right). Note that recAPPs$\alpha$ treatment shifts the curve to the left, significantly reducing the apparent agonist affinity by about 2-fold ($EC_{50}$ of 40.3 ± 6.0 µM, green curve vs. $EC_{50}$ of 80.5 ± 12.7 µM, black curve; n=5; *p<0.05). For statistical evaluation of potentiation we performed a paired two-tailed Students t-test of the agonist-induced currents in the absence and presence of the indicated peptide. n=number of oocytes. Data represent mean ± SEM. $^{ns}p>0.05$, *p<0.05.

**Expanded View Legends**

**EV1: AVV-Venus and AVV-APPs vectors mediate comparable neuron specific expression throughout the dorsal hippocampus**. **A** Double immunostaining of CA1 pyramidal cells. APPsβ (HA-tag, red) is exclusively expressed in neurons (NeuN, blue, left), but not in astrocytes (GFAP, blue, middle) or microglia (Iba1, blue, right). Scale bar: 20 µm. **B** Expression was investigated by immunohistochemistry in different coronal sections from dorsal to ventral. **C** Venus, APPsα and APPsβ (HA-tag) expression is shown for different sections of the hippocampus. Expression is absent in ventral portions of the hippocampus. Scale bar: 1 mm.

**Figure EV2: APPsα and APPsβ are transported through the secretory pathway and secreted efficiently. A** Western blot analysis of lysate (L) and supernatant (SN) of pAAV-APPsα and pAAV-APPsα transfected HEK293T cells using an HA-tag specific antibody. Note the secretion of HA-APPsα and HA-APPsβ into the cell supernatant. Mature APPs is posttranslationally modified and runs at slightly higher MW. **B** Western blot analysis of medium from cortical neurons transduced with AAV-APPsα or AAV-APPsβ. $10^5$ cDKO neurons were transduced at DIV7 with $10^4$ genome copies per cell of either AAV-APPsα or AAV-APPsα. After 12 days medium was exchanged and neuronal supernatants were harvested on the next day. Equal amounts (2 µl) of supernatant were subjected to capillary electrophoresis followed by immunodetection (WES system, ProteinSimple, USA). Note that similar amounts of of HA-APPsα and HA-APPsβ are secreted into the medium of transduced neurons. **C-E** Immunocytochemistry of neurons transduced with AAV-APPsα or AAV-APPsβ neurons. Transduced neurons positive for the neuronal marker NeuN (red) were identified by Venus expression (green). **D, E** Confocal images of neurons stained with the ER marker Calreticulin (green), the Golgi marker Giantin (green) and α-HA staining to detect APPs (red). Note that both HA-APPsα and HA-APPsβ show co-localization (white arrows) with the ER (**D)** and Golgi markers (**E**) indicating successful transport through the secretory pathway. Scale bars: 100 µm (**C**), 10 µm (**D,E**).

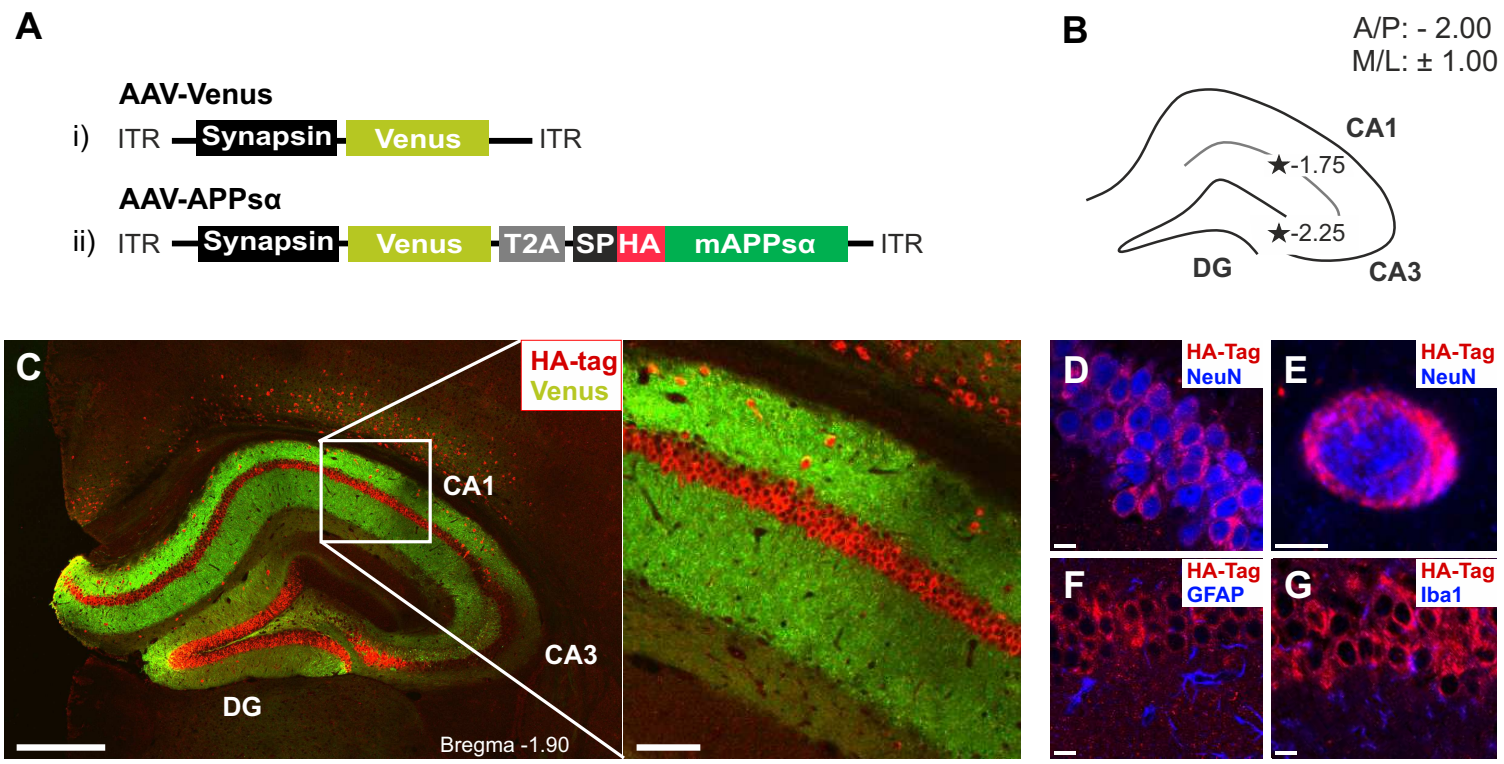
**EV3: Basal transmission in cDKO mice treated with APPsα or APPsβ is not altered compared to LM control animals injected with Venus.**

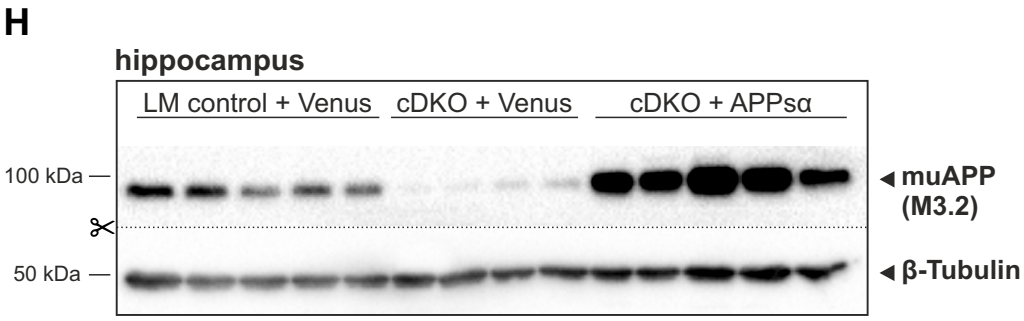
**A, C** Neuronal excitability is comparable at all stimulus intensities (25-250 µA) between all groups injected with the indicated AAV vectors. **B, D** No significant alterations can be detected when analyzing the Input-Output (IO) strength of the viral vector injected groups of mice at any fiber volley (FV) amplitude.

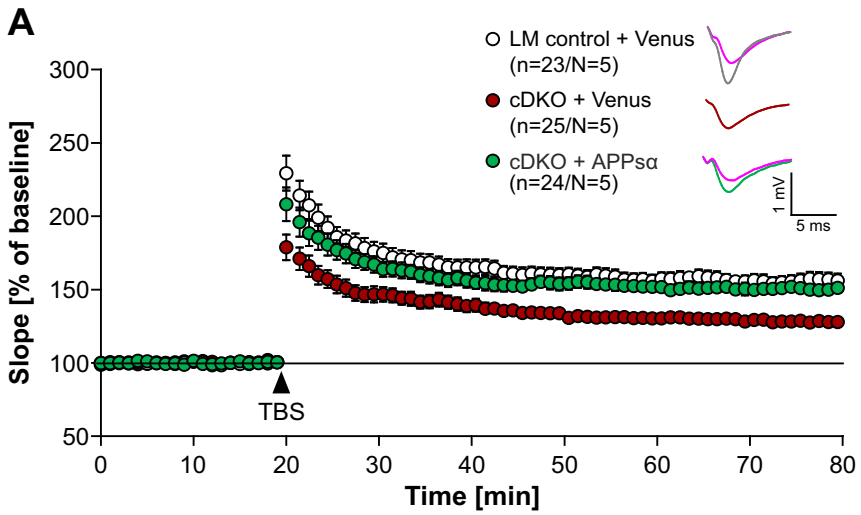
**EV4: Inhibition of α7-nAChRs by BTX after APPsα pre-incubation does not inhibit LTP A** Experimental set-up: pre-application of APPsα or CTα16 before addition of 10 mM Bungarotoxin (BTX) 30 min prior to TBS (arrowhead). **B, C** Hippocampal acute slices treated with APPsα for 1 h and subsequent addition of 10 nM BTX did not significantly impair induction (t20-25) and maintenance of LTP (t75-80) in comparison to slices of cDKO mice recorded in the presence of APPsα alone.

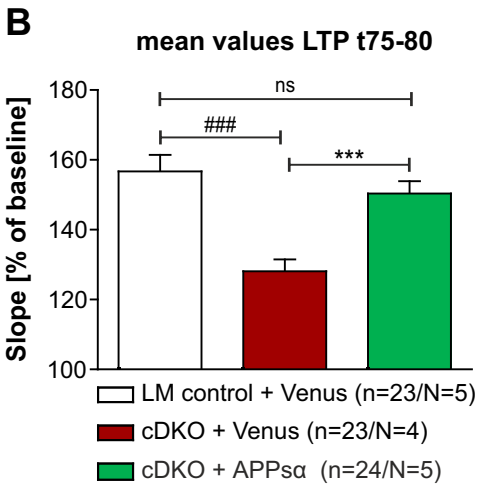
**D, E** The treatment with CTα16 for 1 h and subsequent addition of 10 nM BTX did not significantly impair induction (t20-25) or maintenance of LTP (t75-80). Age: 5-6 months of age. n=number of recorded slices, N=number of animals. Data represent mean ± SEM. Data were analysed by unpaired two-tailed Students t-test. $^{ns}p>0.05$.

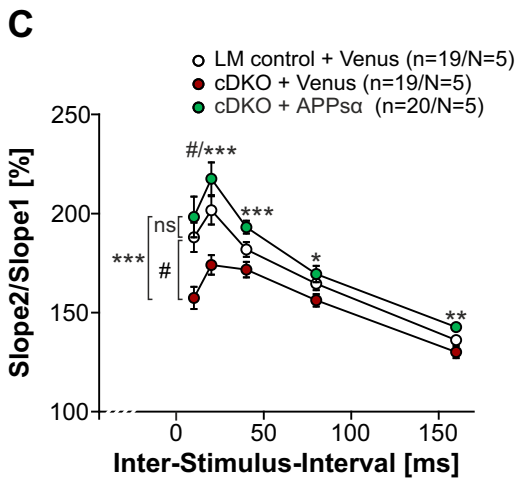
**EV5: Neither CTα16 nor APPsα potentiate the homomeric α1 glycine receptor.**
**A** Glycine-induced whole-cell current traces of homomeric α1 glycine receptor (GlyR) expressed in *Xenopus* oocytes recorded in the absence or presence of CTα16 or APPsα, respectively. No direct activation of α1-GlyR by CTα16 or APPsα in the presence of the agonist was detected (not shown). Bars indicate application of $EC_{50}$ glycine concentrations and of the peptide indicated. **B** Quantification of potentiation shows that neither CTα16 nor APPsα effect glycine-induced currents. Data represent mean ± SEM. Data were analysed by paired two-tailed Students t-test. $^{ns}p>0.05$.

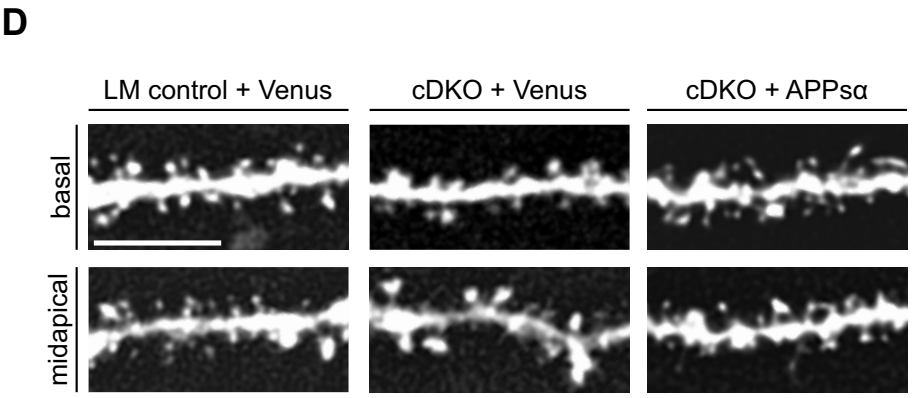

A
AAV-Venus
i) ITR Synapsin Venus ITR
AAV-APPsα
ii) ITR Synapsin Venus T2A SP HA mAPPsα ITR
B
A/P: - 2.00
M/L: ± 1.00
CA1
-1.75
-2.25
DG
CA3
C
HA-tag
Venus
CA1
CA3
DG
Bregma -1.90
D
HA-Tag
NeuN
E
HA-Tag
NeuN
F
HA-Tag
GFAP
G
HA-Tag
Iba1

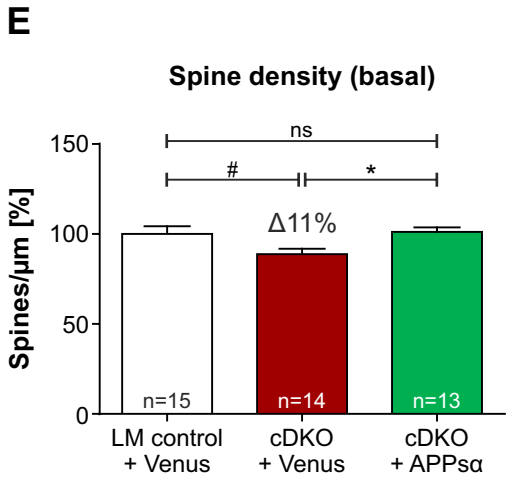

H
hippocampus
LM control + Venus
cDKO + Venus
cDKO + APPsα
100 kDa
50 kDa
muAPP
(M3.2)
β-Tubulin

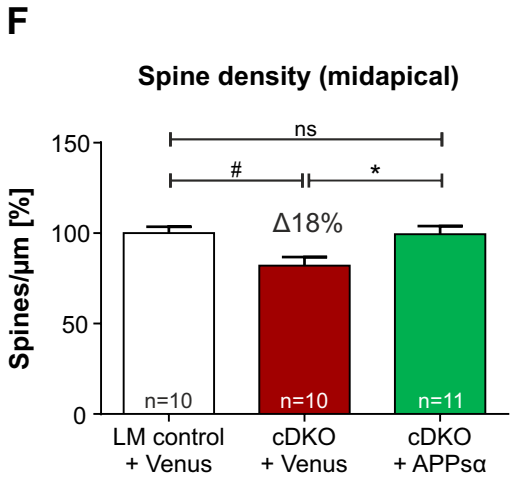

A
LM control + Venus
(n=23/N=5)
cDKO + Venus
(n=25/N=5)
cDKO + APPsα
(n=24/N=5)
1 mV
5 ms
Slope [% of baseline]
TBS
Time [min]

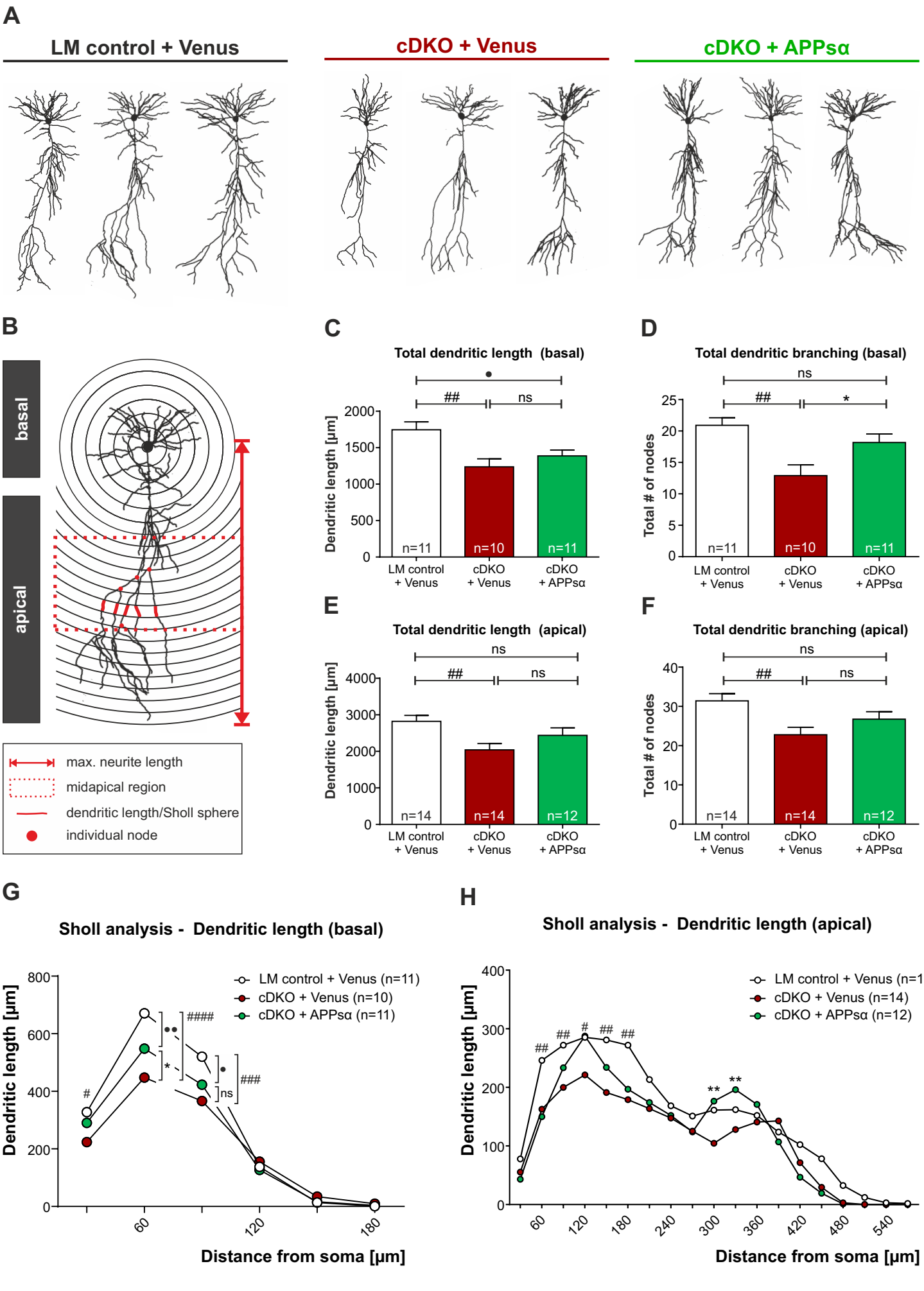

B
mean values LTP t75-80
Slope [% of baseline]
ns
###
***
LM control + Venus (n=23/N=5)
cDKO + Venus (n=23/N=4)
cDKO + APPsα (n=24/N=5)

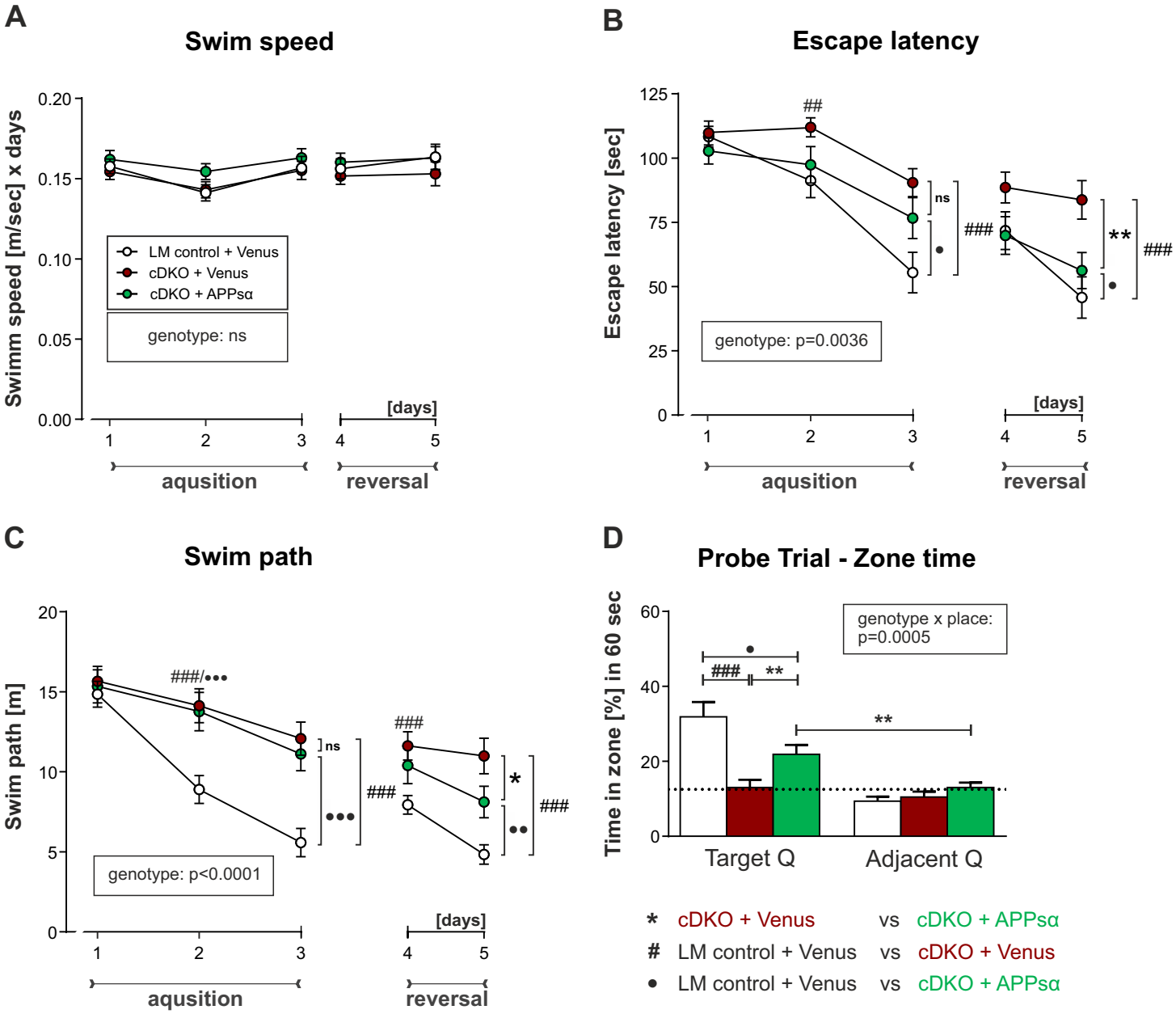

C
LM control + Venus (n=19/N=5)
cDKO + Venus (n=19/N=5)
cDKO + APPsα (n=20/N=5)
Slope2/Slope1 [%]
Inter-Stimulus-Interval [ms]

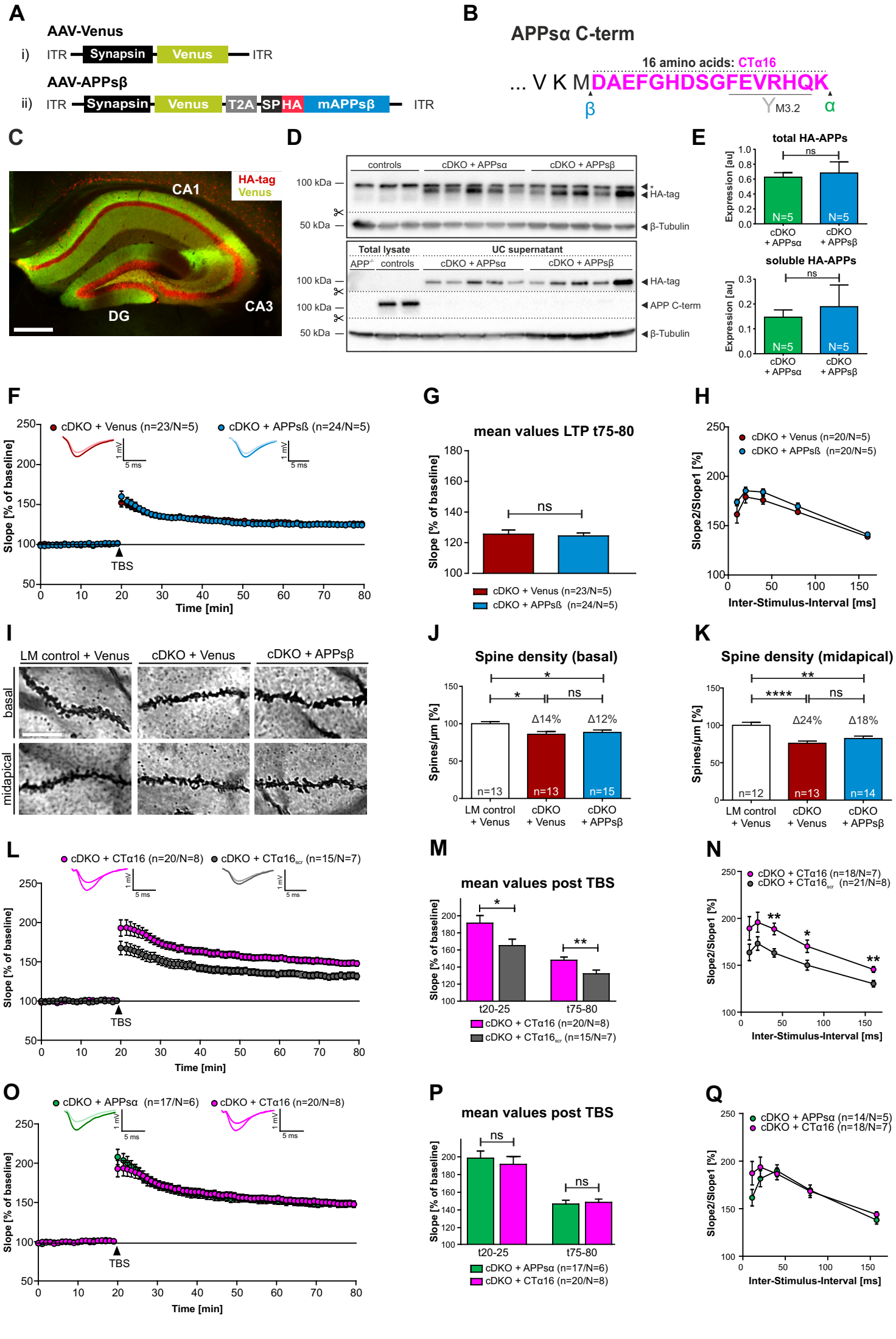

D
LM control + Venus
cDKO + Venus
cDKO + APPsα
basal
midapical

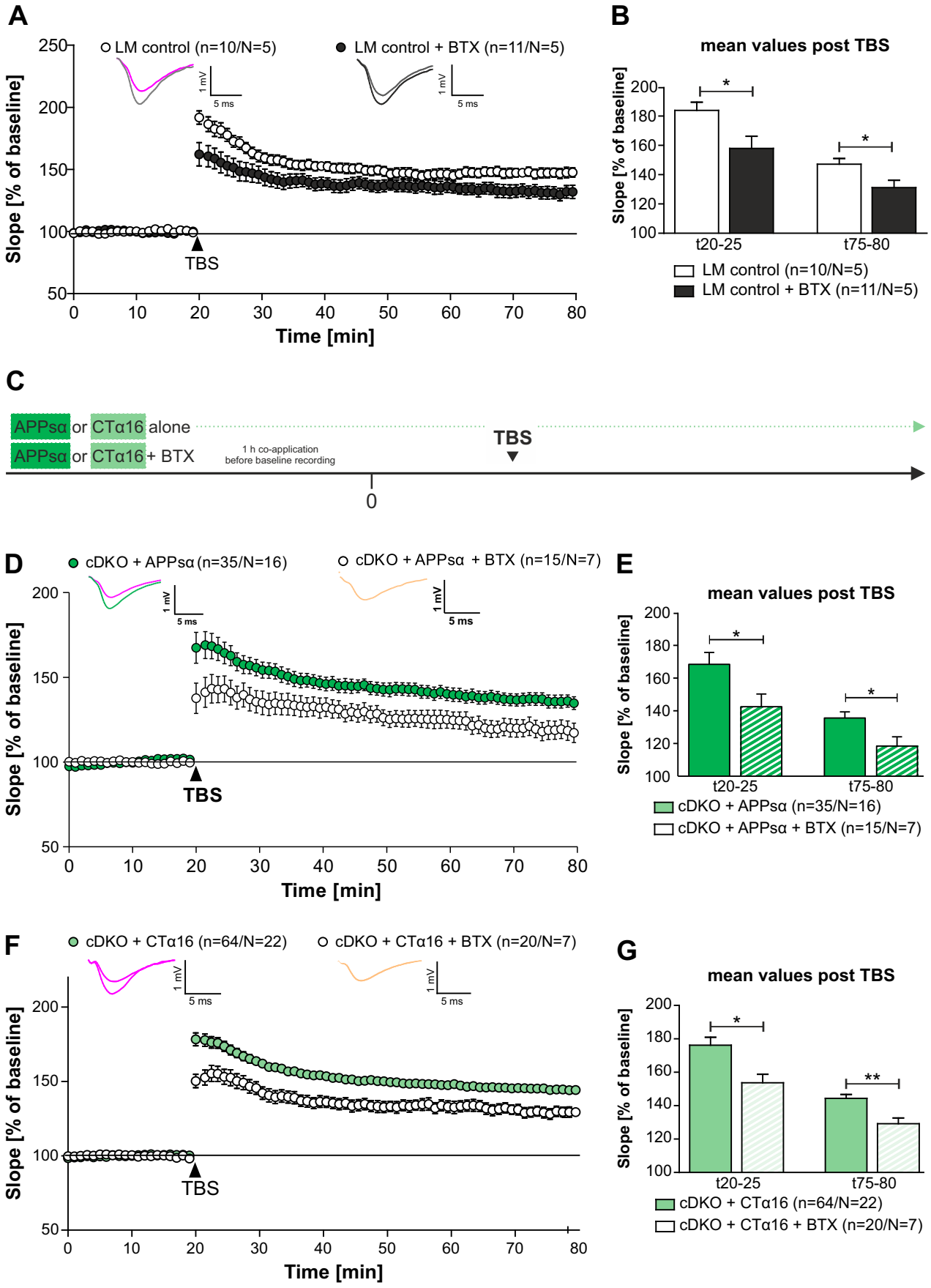

E
Spine density (basal)
Spines/µm [%]
Δ11%
n=15
n=14
n=13
LM control + Venus
cDKO + Venus
cDKO + APPsα

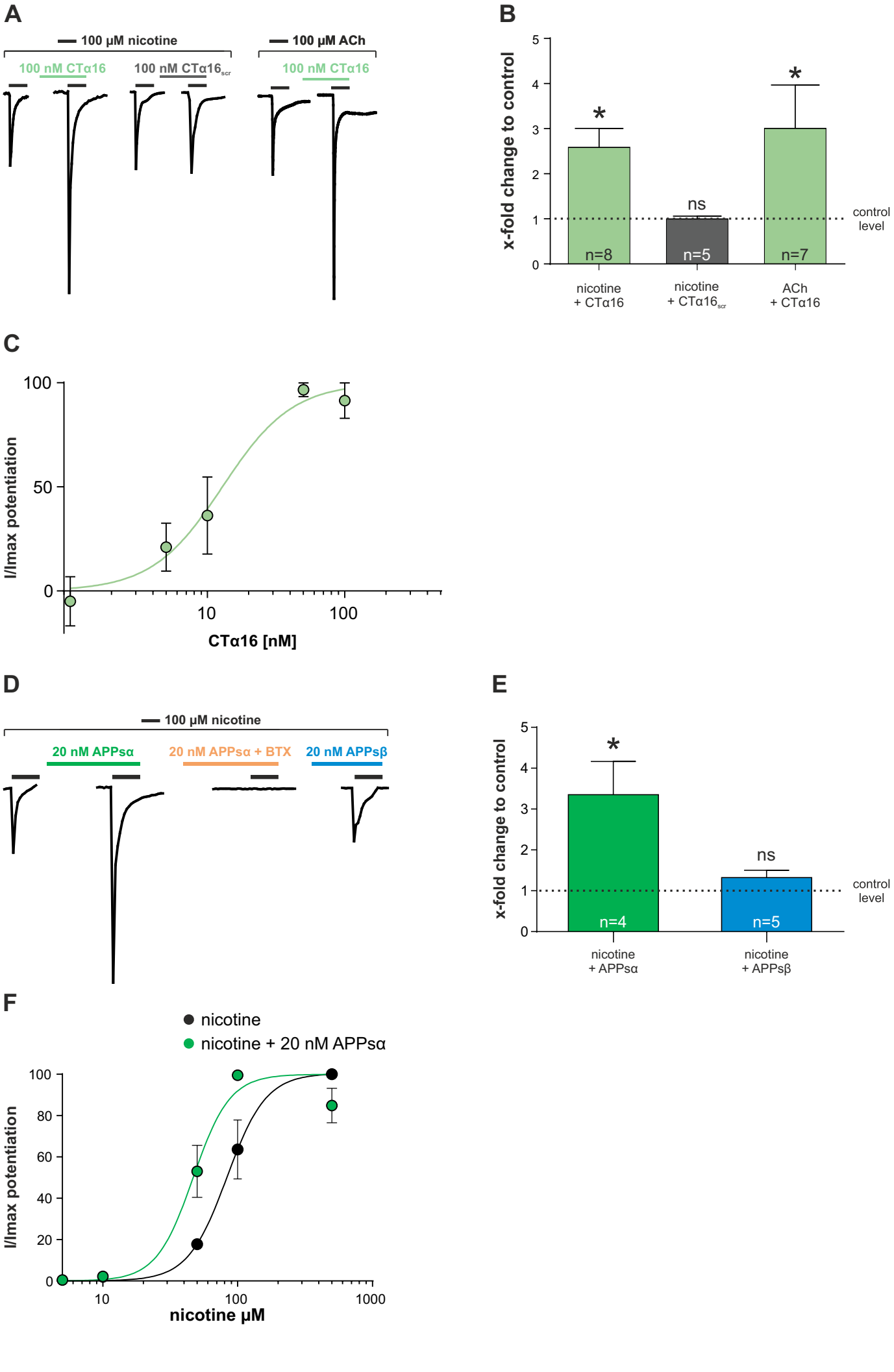

F
Spine density (midapical)
Spines/µm [%]
Δ18%
n=10
n=10
n=11
LM control + Venus
cDKO + Venus
cDKO + APPsα


A
LM control + Venus
cDKO + Venus
cDKO + APPsα
B
basal
apical
max. neurite length
midapical region
dendritic length/Sholl sphere
individual node
C
Total dendritic length (basal)
Dendritic length [µm]
n=11
n=10
n=11
LM control + Venus
cDKO + Venus
cDKO + APPsα
D
Total dendritic branching (basal)
Total # of nodes
E
Total dendritic length (apical)
n=14
n=14
n=12
F
Total dendritic branching (apical)
G
Sholl analysis - Dendritic length (basal)
LM control + Venus (n=11)
cDKO + Venus (n=10)
cDKO + APPsα (n=11)
Distance from soma [µm]
H
Sholl analysis - Dendritic length (apical)
LM control + Venus (n=14)
cDKO + Venus (n=14)
cDKO + APPsα (n=12)
Distance from soma [µm]


A
Swim speed
Swimm speed [m/sec] x days
0.20
0.15
0.10
0.05
0.00
LM control + Venus
cDKO + Venus
cDKO + APPsα
genotype: ns
[days]
1
2
3
4
5
aqusition
reversal
B
Escape latency
Escape latency [sec]
125
100
75
50
25
0
##
ns
•
###
**
genotype: p=0.0036
C
Swim path
Swim path [m]
20
15
10
5
0
###/•••
ns
•••
###
*
••
genotype: p<0.0001
D
Probe Trial - Zone time
Time in zone [%] in 60 sec
60
40
20
0
genotype x place:
p=0.0005
Target Q
Adjacent Q
* cDKO + Venus vs cDKO + APPsα
# LM control + Venus vs cDKO + Venus
• LM control + Venus vs cDKO + APPsα


A
AAV-Venus
i) ITR Synapsin Venus ITR
AAV-APPsβ
ii) ITR Synapsin Venus T2A SP HA mAPPsβ ITR
B
APPsα C-term
16 amino acids: CTα16
... V K M DAEFGHDSGFEVRHQK
β
γ M3.2
α
C
HA-tag
Venus
CA1
CA3
DG
D
controls
cDKO + APPsα
cDKO + APPsβ
100 kDa
50 kDa
HA-tag
β-Tubulin
Total lysate
UC supernatant
APP-/-
APP C-term
E
total HA-APPs
soluble HA-APPs
Expression [au]
ns
N=5
cDKO + APPsα
cDKO + APPsβ
F
cDKO + Venus (n=23/N=5)
cDKO + APPsß (n=24/N=5)
Slope [% of baseline]
TBS
Time [min]
G
mean values LTP t75-80
ns
H
cDKO + Venus (n=20/N=5)
cDKO + APPsß (n=20/N=5)
Slope2/Slope1 [%]
Inter-Stimulus-Interval [ms]
I
LM control + Venus
cDKO + Venus
cDKO + APPsβ
basal
midapical
J
Spine density (basal)
Spines/µm [%]
Δ14%
Δ12%
n=13
n=13
n=15
LM control + Venus
cDKO + Venus
cDKO + APPsβ
K
Spine density (midapical)
Δ24%
Δ18%
n=12
n=13
n=14
L
cDKO + CTα16 (n=20/N=8)
cDKO + CTα16scr (n=15/N=7)
M
mean values post TBS
t20-25
t75-80
N
cDKO + CTα16 (n=18/N=7)
cDKO + CTα16scr (n=21/N=8)
O
cDKO + APPsα (n=17/N=6)
cDKO + CTα16 (n=20/N=8)
P
mean values post TBS
Q
cDKO + APPsα (n=14/N=5)
cDKO + CTα16 (n=18/N=7)


A
LM control (n=10/N=5)
LM control + BTX (n=11/N=5)
1 mV
5 ms
TBS
Slope [% of baseline]
Time [min]
B
mean values post TBS
t20-25
t75-80
LM control (n=10/N=5)
LM control + BTX (n=11/N=5)
C
APPsα or CTα16 alone
APPsα or CTα16 + BTX
1 h co-application
before baseline recording
TBS
0
D
cDKO + APPsα (n=35/N=16)
cDKO + APPsα + BTX (n=15/N=7)
E
cDKO + APPsα (n=35/N=16)
cDKO + APPsα + BTX (n=15/N=7)
F
cDKO + CTα16 (n=64/N=22)
cDKO + CTα16 + BTX (n=20/N=7)
G
cDKO + CTα16 (n=64/N=22)
cDKO + CTα16 + BTX (n=20/N=7)


A
100 µM nicotine
100 µM ACh
100 nM CTα16
100 nM CTα16scr
100 nM CTα16
B
x-fold change to control
control level
ns
n=8
n=5
n=7
nicotine + CTα16
nicotine + CTα16scr
ACh + CTα16
C
I/Imax potentiation
CTα16 [nM]
D
100 µM nicotine
20 nM APPsα
20 nM APPsα + BTX
20 nM APPsβ
E
x-fold change to control
control level
ns
n=4
n=5
nicotine + APPsα
nicotine + APPsβ
F
nicotine
nicotine + 20 nM APPsα
I/Imax potentiation
nicotine µM